Combined Therapy for Non-Resectable Squamous Cell Carcinoma of the Oral Cavity

M.F. Noorman van der Dussen

Combined Therapy for Non-Resectable Squamous Cell Carcinoma of the Oral Cavity.

Local Selective Intra-arterial Infusion with Adriamycin and Bleomycin Combined Concurrently with Radiotherapy. The Predictive Value of Flow Cytometry in Monitoring Cell Cycle Kinetic Effects of Therapy

Gecombineerde behandeling van niet resectabele plaveiselcel-carcinomen van de mondholte.

(met een samenvatting in het Nederlands)

PROEFSCHRIFT

Ter verkrijging van de graad van doctor in de geneeskunde
aan de Rijksuniversiteit te Utrecht,
op gezag van de Rector Magnificus Prof. dr. J.A. van Ginkel,
volgens besluit van het College van Dekanen
in het openbaar te verdedigen op dinsdag 30 september 1986
des namiddags te 2.30 uur.

door

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geboren op 5 juli 1942 te Bandung, Java.

Drukwerkverzorging OPTIMAX
Wijk bij Duurstede

Promotores:

Prof. dr. P. Egyedi
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Stellingen behorende bij het proefschrift " Combined Therapy for Non-resectable Squamous Cell Carcinoma of the Oral Cavity".

M.F. Noorman van der Dussen
Utrecht, 30 september 1986.

1. DNA-flowcytometrie gedurende gecombineerde chemo- en radiotherapie van niet resectabele mondholte carcinomen, geeft een aanwijzing of curatie tot de mogelijkheden behoort.
   (Dit proefschrift).

2. Een plaveiselcelcarcinoom van de mondholte met doorgroei in het bot, is niet te cureren door chemo- en/of radiotherapie alleen.
   (Dit proefschrift).

3. Het gegeven "partiële remissie" na inductie chemotherapie heeft waarschijnlijk geen betekenis voor de overleving.

4. Een patient met een invasief tong/mondbodem carcinoom heeft recht op chirurgie van de hals.

5. Het verslavingsgedrag ten aanzien van roken wordt zelfs na een zeer belastende combinatie behandeling niet of nauwelijks in positieve zin beïnvloed.
   (Dit proefschrift).

6. Bij een gecureerde tumor van de bovenste lucht/voedselweg is het stoppen met roken een waarschijnlijk effectievere adjuvante maatregel dan het toedienen van adjuvante chemotherapie.
   (John Cairus, Scientific American, november 1985).

7. Veel radiotherapie schema's zijn meer afhankelijk van het aantal werkdagen in een week, dan dat ze gebaseerd zijn op celkinetische parameters van de behandelde tumor.
   (Poulakos et al., J. Nat. Cancer Inst. 1975).

8. Orthognathische chirurgie, in het bijzonder van schisispatienten is suboptimaal zonder medewerking van een agressieve specialist voor dentomaxillaire orthopedie.

9. De verschillende chirurgische augmentatie technieken ter correctie van de atrofische mandibula volgen elkaar in hoog tempo op; dit bewijst dat geen van deze operatieve methoden konden voldoen aan de hooggespannen verwachtingen waarmee zij werden geïntroduceerd.

10. Het behandelen van patienten in teamverband houdt niet automatisch in dat een patient ook beter behandeld wordt.
(Egyedi, inaugurele rede 1978).

11. De vrouw/man verhouding van universitaire hoofddocenten is voor de R.U.U. 1:20 (U-blad 24januari 1986).
Deze verhouding valt niet te verklaren uit de I.Q.-ratio van de geslachten.

12. Anarchie, gestoeld op welke ideologie dan ook, heeft op de samenleving dezelfde invloed als een maligne tumor op het menselijk lichaam en ondergaat hetzelfde lot; wanneer de maatschappij door haar destructieve krachten te gronde gaat sterft wegens haar parasitaire karakter de anarchie eveneens.

13. Het is zorgwekkend dat binnen academische ziekenhuizen binnenkort meer inkt dan bloed vloeit.

14. Niet de getalenteerde deskundige, maar degene die het beste de deur van de minister weet te vinden, maakt in het burocratisch formalistisch (inter)universitair bestel de dienst uit. Een tandheelkundige traditie is daardoor voor ons land verloren gegaan.

Ter nagedachtenis aan mijn grootmoeder.
1893 - 1985

Voor Betty en de kinderen

Dit proefschrift kwam mede tot stand dank zij de financiële steun van de Utrechtse Stichting tot Bevordering van de Mondheelkunde; de firma H. Lundbeck & Co. A/S.

CONTENTS

INTRODUCTION

Improved operative and radiotherapeutic techniques or combinations of both, with or without adjuvant chemotherapy, have considerably improved the regional cure of head and neck tumours in the past decade.

In the case of higher staged tumours, the ceiling of improved survival percentages has been reached. The optimal treatment for a patient with a head or neck tumour is that which offers the greatest chance of cure, maintaining the most satisfactory physiological function and cosmetic appearance, such that the patient is again able to take his place in society (Jesse, 1981). In Stage IV squamous cell carcinoma (UICC, 1978; AJC, 1978), surgery gives slight chances of regional cure because of the proximity of vital structures. The required heroic procedures have lead to substantial mutilation. Radiotherapy also rarely results in cure, one reason being that bone invasion has almost always occurred with these large tumours. Chemotherapy alone results only seldom in a permanent cure in small tumours. Other ways must therefore be sought to offer these patients a better chance.

When considering one or more different therapy modes, better prognoses in the treatment of Stage IV head and neck tumours can probably be achieved if sufficient information is available concerning the expected reaction of the individual tumour to the therapy employed. The need to find biological parameters which together with clinical and histological parameters can give a prognosis with regard to the chances of regional cure in treatments which require as much of the patient as the combination therapy which is detailed in this dissertation, was the stimulant for this work.

There have been other attempts to carry out initial prognostic examination. We refer to the research with human xenografts on naked mice (Braakhuis and Snow, 1984), the purpose of which was to predict the effect of the chemotherapy employed on individual tumour behaviour. Colony-forming assays should provide information concerning the chemotherapeutic agents which will produce a tumour response. The time required to culture individual tumour cells and the low percentage of successful cultures, makes this method unsuitable for clinical use (Salmon, 1980). Neither does it appear that there is a good correlation between the clinical response of the tumour and the in vitro result. In general, the prediction tests of response to particular chemotherapeutic agents are not yet sufficiently refined to be practically useful (Chabner et al., 1984).

A promising way to acquire information about individual

tumour behaviour, would seem to be the determination of cell kinetic properties (Barlogie et al., 1982) and, even more importantly, the changes they undergo during treatment (Silvestrini et al., 1984). Ideally, knowledge of changes in cell cycle at all times would be desirable so that optimal regulation of the chosen chemotherapy and/or radiotherapy could lead to maximal cell death of the tumour cells with minimal toxicity for the host cells. A possible gain for increasing insight into the characteristics of a particular tumour is DNA flow cytometry. By taking multiple biopsies before and during the chemotherapy/radiotherapy treatment, it is perhaps possible to predict the expected clinical results. Research relating to this is described in this dissertation: 16 patients with non-resectable tumours of the oral cavity were treated with concurrent intra-arterial chemotherapy and radiotherapy. Multiple biopsies were analyzed with DNA flow cytometry and an attempt was made to correlate the clinical response with the acquired cellular kinetic data.

The purpose of the research can thus be summarized as:

1. Is regional cure of non-resectable squamous cell carcinomas possible using the method described?
2. Is it possible, using DNA flow cytometry, to predict the chance of successful therapy?

PART I

GENERAL INFORMATION

CHAPTER I

Oral Cavity Carcinoma

1. Oral cavity carcinoma.

Three to 5% of all malignant disorders occur in the oral cavity. The vast majority of oral cavity neoplasms are squamous cell carcinomas: Batsakis (1974) records 90%, Bhaskar (1969) even more than 90%. The percentages for carcinoma of the naso-pharynx and the maxillary sinus are respectively 58% and 51% (Powell and Robin, 1983). In the mobile, anterior 2/3 of the tongue, 99% of malignant tumours were squamous cell carcinomas (Strong and Spiro, 1981). This percentage decreases in other locations: floor of the mouth - 98%, gingiva - 93%, buccal mucosa - 84%, hard palate - 48%.

In most populations studied, the squamous cell carcinoma occurs in the fourth decade of life with a peak in the sixth decade. Few carcinomas occur in patients under 20 years of age. In a percentage distribution of locations within the oral cavity, Tieke and Bernier (1954) recorded: tongue - 52%, floor of the mouth - 16%, alveolar mucosa - 12 %, palate - 11%, buccal mucosa - 9%. Bhaskar (1969) found 50% in the tongue, 16% in the floor of the mouth and 34% in other locations. From the records (1966 - 1974) in the Memorial Hospital in New York City, Strong and Spiro (1981) recorded a more even distribution of tongue and floor of the mouth carcinomas. They found more than 33% of the carcinomas on the tongue, followed directly by carcinomas of the floor of the mouth.

2. Incidence.

There are some geographical differences with regard to the incidence of oral cavity carcinoma. In a country such as India, oral cavity carcinoma is one of the most prevalent carcinomas, while in north-west Europe it can be called a relatively rare tumour. In general, it can be said that the male/female ratio is 1:1, but this figure varies somewhat. In The Netherlands the incidence of tongue carcinoma is 1 - 2 per 100000 per year (Oldhoff et al., 1973). Waterhouse et al. (1982) recorded, for instance, very high incidences in Bombay and Puerto Rico, 10.2 and 6 respectively, and in the French provinces of Bas-Rhin and Doubs, 7.6 and 7.8 respectively.

As far as the male/female ration is concerned, the figures of Powell and Robin (1983) from the West Midlands (England) and from Steensma (1971), (the Netherlands) can be quoted. They found a ratio of 1.9 and 1.08 respectively. Finally, it can be stated that the other locations in the oral cavity run parallel with those of the tongue.

3. Aetiological factors.

Specific aetiological factors can be identified from

epidemiological research. As regards tongue and floor of the mouth carcinoma, the combination of tobacco and alcohol as aetiological factors has been made plausible (Waterhouse et al., 1982).

In the French provinces of Bas-Rhin and Doubs, heavy tobacco and highly concentrated alcohol (distilled) are used significantly more intensively than in comparable French provinces. However, in our own patient material, the number of older women who have never smoked or drunk is considerable. This phenomenon is, of course, well known, and other factors besides tobacco and alcohol have for a long time been seen as possible contributing factors. These are:

1. Inflammation
   i. Chronic aspecific inflammatory disease: (periodontal diseases, dental granulomas, poor oral hygiene).
   ii. Specific inflammatory disease: (candidiasis (Martin, 1940), syphilis).
   iii. Viral (chronic herpes).
2. Avitaminosis
3. Dietary deficiencies
4. Anaemia (Plummer-Vinson)

The role of heriditary factors is still unproven, but there are indications that genetic deficiencies in the multi-factorial process or carcinogenesis are present (v.d. Waal and v.d. Kwast, 1981).

4. Clinical staging of oral cavity tumours.

4.1. Introduction.

A fundamental necessity for every form of clinical therapeutic cancer research is the creation of homogeneous patient groups

1. to be able to compare the results of different therapies
2. to determine the prognosis of the treatment
3. for the purpose of therapy choice
4. for the communication between the different specialists involved in treatment.

The most frequently used classification methods at the present time are those of the American Joint Committee (AJC, 1978) and those of the Union Internationale Contre le Cancer (UICC, 1978).

4.2. Tumour (T) Classification Using the TNM System.

The tumour classification systems of the UICC (1978) and AJC (1978) are almost identical:

Tis: Pre-invasive carcinoma (carcinoma in situ)

T_0 : no evidence of primary tumour
T_1 : tumour 2 cm or less in its greatest dimension
T_2 : tumour more than 2 cm but not more than 4 cm in its greatest dimension
T_3 : tumour more than 4 cm in its greatest dimension
T_4 : tumour with extension to e.g. bone, muscle, skin, antrum, neck
T_x : the minimum requirements to assess the primary tumour can not be met.

The difficulty with evaluation of the primary tumour, even for experienced investigators in optimal conditions (such as staging under anaesthesia), is the high degree of subjectivity which leads to inaccuracies. The Dösak* classification still follows the UICC (1973) scheme for the primary tumour, because bone invasion of relatively small floor of the mouth and gingival tumours should not be classified as T_4 for prognostic purposes.

4.3. Regional Lymph Node (N) Classification Using the TNM System

The lymph node classification is clearly different in the UICC (1978) and the AJC (1978) systems. The UICC focuses mainly on the degree of attachment and location of the lymph nodes, and AJC concentrates primarily on size, number and location of the lymph nodes in the neck:

UICC (1978)
N_0 : No evidence of regional lymph node involvement
N_1 : Evidence of involvement of moveable homolateral regional lymph nodes
N_2 : Evidence of involvement of moveable contralateral or bilateral regional lymph nodes
N_3 : Evidence of involvement of fixed regional lymph nodes
N_x : The minimum requirements to assess the regional lymph nodes can not be met.

AJC (1978)
N_x : Nodes can not be assessed
N_0 : No clinically positive node
N_1 : Single clinically positive homolateral node 3 cm or less in diameter
N_2 : Single clinically positive homolateral node more than 3 cm but not more than 6 cm in diameter
N_2a : Single clinically positive homolateral node more than 3 cm but not more than 6 cm in

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* Deutsch - Osterreichisch-Schweizerischer Arbeitskreis fur Tumoren im Kiefer - und Gesichts - bereich (German - Austrian - Swiss Association for Head and Neck Tumours).

diameter

N_2b : Multiple clinically positive homolateral nodes, none more than 6 cm in diameter

N_3 : Massive homolateral node(s), bilateral nodes, or contralateral node(s)

N_3a : Clinically positive homolateral node(s), one more than 6 cm in diameter

N_3b : Bilaterally clinically positive nodes (in this situation, each side of the neck should be staged separately; that is N_3b; right, N_2a; left, N_1)

N_3c : Contralateral clinically positive node(s) only.

At the International Conference on Head and Neck Cancer in Baltimore, Baker (1984) announced that in 1986 a new TNM classification, which is the result of discussions between the AJC and the UICC, will almost certainly be introduced. The T and M classifications will remain unchanged but the N classification will be as follows:

N_x : Minimum requirements to assess the regional node can not be met

N_0 : No clinically positive node

N_1 : Single clinically positive homolateral node, 3 cm or less in diameter.

N_2 : Single clinically positive homolateral node, more than 3 cm but not more than 6 cm in diameter, or multiple clinically positive homolateral nodes, none more than 6 cm in diameter, or clinically positive bilateral or contralateral nodes, none more than 6 cm in diameter. (This N_2 category can be broken down into N_2a, N_2b, and N_2c)

N_3 : Clinically positive homolateral node(s), one more than 6 cm in diameter.

Thus, we see here an obvious combination of both of the existing N classifications. In clinical staging it is thus important to accurately describe tumour and lymph nodes with regard to localization, degree of infiltration, size and also the relationship to surrounding structures. This offers the advantage that with changes in classification system, the data can be reclassified.

4.4. Distant Metastasis (M) Classification Using the TNM System.

M_0 : No evidence of distant metastasis

M_1 : Evidence (clinical, radiological, histopathological) of distant metastasis

The TNM classification can by way of combination, produce a level of classification in which:

Stage I : T_1, N_0, M_0

Stage II : T_2, N_0, M_0

Stage III : T_3, N_0, M_0
$T_1, T_2, T_3; N_1; M_0$
Stage IV : T_4, N_0, M_0
$T_1, T_2, T_3, T_4; N_2, N_3; M_0$
$T_1, T_2, T_3, T_4; N_0, N_1, N_2, N_3; M_1$

4.5. The TNM System as a Prognostic Indicator and the Therapy-Dependent Prognostic Index (TPI) (Platz et al., 1983).

The combination of in-built errors within tumour classification systems are sufficient to explain the best and worst results of many published cure rates without consideration of factors concerning the quality of the adopted therapy (Harrison, 1978, 1979). It also appears from the investigations of Dösak (Platz et al., 1982, 1983), that the TNM system does not offer sufficient guarantee for creating homogeneous patient groups.

In a retrospective analysis of 1021 patients with a carcinoma of .the oral cavity, lip and oropharynx from 13 clinics in Germany, Austria and Switzerland, 18 clinically relevant factors pertaining to the first examination were analyzed for their prognostic significance by both univariate and multivariate procedures. Only 7 of these 18 clinically relevant factors proved to be of prognostic relevance:

1. the tumour size
2. the degree of infiltration of the tumour (histologically verified)
3. the degree of histological differentiation
4. the localization of the primary tumour
5. evidence of enlargement, clinical aspect, degree of fixation of regional lymph nodes
6. evidence of distant metastasis
7. the age of the patient

On the basis of these multivariate analyses, a prognostic index was established.

Four variables were introduced for the T classification: two for the tumour size (less than 4 cm or greater than 4 cm), and two for the tumour infiltration (greater or less than 5mm).
Two variables were introduced for the N classification: clinically positive and fixed lymph nodes, or all other N stages.
Two variables were introduced for the M classification: evidence or no evidence of distant metastasis.
Three variables were introduced for age: younger than 50 years, between 50 and 70 years, older than 70 years.

On the basis of the high statistical significance of some therapy-dependent factors, a prognostic index without the

introduction of these relevant therapy details is incomplete and unacceptable. This led (Platz et al., 1982) to a therapy-dependent prognostic index (TPI). The 4 prognostically relevant therapy-dependent factors comprised:

1. Extensive radical surgery cases
2. Non-radical surgery cases, palliative radiated cases, or patients who underwent alternative therapy.
3. No evidence of tumour after completion of treatment.
4. Evidence of remaining tumour after completion of treatment.

With the establishment of a TPI (therapy-dependent prognosis index) according to the Dösak study, more homogeneous patient groups can probably be compiled than by using only the TNM classification method. Results of adjuvant therapies combined with existing treatment modalities can be better evaluated.

We could thus perhaps question the speculations of Andrew Lane (1905) (cited by Harrison, 1983) with regard to the use of statistics on relatively small groups of patients with many variables: "We use statistics as a drunken man uses lamp-posts: for support rather than illumination". Some criticism must be made of the practice of using tables with therapy-dependent prognostic indices. All forms of indexing carry the risk of 'misinterpretation'. Even in the most optimally established prospective randomized trials, we can demonstrate only small shifts in local and regional cure rates with large numbers of patients in the separate therapy categories. The only correct solution thus lies in a protocol in which as many patients as possible are treated within an integrated, cooperative relationship such as the EORTC and the Dösak (in Europe). Because of the great importance of the relative factors concerning survival and prognosis, further attention is given to this subject in the following paragraph.

5. Relevant factors concerning survival and prognosis in oral cavity carcinoma.

Eighteen relevant factors from the literature which influence the prognosis of oral cavity carcinomas were examined. After multivariate analysis, it appeared that only 7 factors were significantly relevant (Platz et al., 1983), as far as this research is concerned. For the sake of completeness, it is recorded that it was a retrospective study from 13 clinics with all the inaccuracies this could imply. The 7 factors of prognostic relevance are independently discussed.

5.1. Tumour Size.

From clinical evaluation it appears that prognosis becomes worse with increase in tumour size (AJC, 1978; Cachin, 1975; Shear et al., 1976). Crissman et al. (1980) saw in a follow-up study of oral cavity carcinoma a significantly greater cure rate for tumours smaller than 4 cm. In his

patient material, the two year survival rate for tumours classified as T_1 , T_2 and T_3 are respectively 91%, 73% and 50%. In the Dösak study (Platz et al., 1983) it appeared that after a multivariate analysis of oral cavity carcinomas, the prognosis of T_3 (larger than 4 cm) tumours was very much worse than the prognosis for T_2 (2 - 4 cm) tumours. There appeared to be no significant difference between T_1 and T_2 tumours. With regard to prognosis, the T_1 and T_2 oral cavity carcinomas can thus be seen as one group, at least according to the Dösak study.

5.2. Stage of Invasion (and Bone Destruction).

Scanton et al. (1969), Krause et al., (1973) and Applebaum et al., (1980) found a deterioration in the prognosis in oral cavity carcinomas with increase in the depth of invasion. T_1 and T_2 squamous cell carcinomas with only superficial or micro-invasion of the submucosa have little or no propensity for lymph node metastases while T_2 carcinomas with invasion through or into the submucosa, have a substantial frequency of regional metastatic disease (Crissman et al., 1980). Verkerk (1978) found a significant correlation between local cure of floor of the mouth carcinoma and the extent of tumour invasion: in cases with superficial growth, 70% of the patients were tumour-free and in cases of deep invasion, 35%.

In the Dösak (Platz et al., 1983) research, four groups with the following variables were analyzed on a multivariate basis:
1. Superficial tumour invasion (to a maximum of 5 mm)
2. Deep invasion (over 5 mm)
3. Involvement of adjacent structures and organs
4. Uncertain tumour invasion (could not be assessed retrospectively).

When the groups 2, 3 and 4 were combined into a group of non-superficially growing carcinomas, it appeared that this group had a significantly reduced prognosis when compared with the superficially growing carcinomas.

5.3. Histological Differentiation.

The histological classification of Broders (1920) has a much more limited prognostic relevance than has until now been accepted (Platz et al., 1983). The frequency of mitosis is greater for undifferentiated (anaplastic) carcinomas than for the moderately well-differentiated to well-differentiated carcinomas. This is an indication of biological aggression of carcinomas (Crissman et al., 1980) as opposed to the classification of Broders (1920). Platz et al. (1983) differentiates the tumour into three histopathological levels: 1) keratinizing 2) non-keratinizing and 3) anaplastic, and found with multivariate analysis a

significant difference between 1, and 2 and 3. Anaplastic carcinomas appeared to have a worse prognosis.

5.4. Localization of the Primary Tumour.

Tongue carcinoma.

For the mobile (anterior 2/3) part of the tongue, greatly differing 5 year survival percentages are given. The old literature shows very low percentages: Martin et al., (1940) 22%, Gibbel et al., (1949) 14%. With improved therapeutic possibilities, more favourable percentages have been achieved. Steensma (1971) found a 5-year survival rate of 43% in the evaluation of patients of the Rotterdam Radiotherapy Institute, and 37% in the evaluation of the patients of the Antoni van Leeuwenhoek Hospital. The most recent statistics for The Netherlands are even more favourable (between 55 and 60%, van Andel et al., 1983).

Floor of the mouth carcinoma.

Verkerk (1978) in his patient study, registered a 5-year survival rate of 34%. This figure scarcely differs from the tongue carcinoma rate. Better 5-year survival percentages are found for the lower stages (I and II) of carcinoma of the floor of the mouth than for tongue carcinoma. Crissman et al. (1980) suggests that this comes about through a broader resection for floor of the mouth carcinoma at an early stage, where tumour spread is clinically more difficult to determine than in tongue carcinoma.

The most recent 5-year survival rates of floor of the mouth carcinoma in The Netherlands (between 55 and 70%) can be found in the publication of van Andel et al. (1983).

Other localizations.

In the Dösak study (Platz et al., 1983) the localization and survival rates of the primary tumour in various organs of the oral cavity were evaluated. After multivariate analysis, a statistically significant difference between oropharynx and oral cavity carcinoma could not be demonstrated.

It should be noted that in carcinoma of the cheek, the so-called verrucous carcinoma has a distinctly better prognosis than the ulcerating squamous cell carcinoma (v.d. Waal and v.d. Kwast, 1981).

5.5. Regional Metastasis.

5.5.1. Mechanisms of Regional Metastases.

Malignant cells are destroyed in the lymph nodes by

cell-mediated-immune reactions. When a tumour embolism occurs in the lymph drainage system, a compromise of the immune-defense system is a probable reason why the tumour embolism can become established and grow. It is known that the incidence of malignant (virus induced) tumours in patients receiving immuno-suppressive therapy can be a hundred times that of age-matched controls (Hoover and Fraumeni, 1973).

5.5.2. Regional Metastasis Pattern and Primary Tumour Localization.

The tongue/floor of the mouth carcinoma presents, because of the anatomy of the lymph drainage system, a greater risk to the patient. Tumours in the mid-line can metastasize bilaterally and to lymph nodes lower in the neck, even when the submandibular lymph nodes are negative. Rouviere (1938) performed an anatomical study of the lymph drainage system of the tongue, floor of the mouth and neck, and clarified the above-mentioned metastasis patterns.

Steensma (1971) found clinically positive lymph nodes in 18% of tongue tumours. The distribution was as follows: 79% ipsilateral, 20% bilateral, 1% contralateral. In a distribution according to level, he found 36% subdigastric, directly followed by 31% submandibular. A not important percentage of 18% was found at the mid-cervical level. The other levels contain clinically positive lymph nodes much less often.

The metastasis frequency of cheek carcinomas is rather high (Vegers et al., 1979). Metastasis occurs in the first instance, to the submandibular (sub-mental) lymph nodes, and later to the lymph nodes along the deep jugular vein.

In sinus, hard palate and soft palate carcinomas, the initial filtering lymph nodes are the parapharyngeal and retro-pharyngeal lymph nodes. This region is not resected in a standard neck dissection, therefore elective cervical lymph node surgery for upper jaw tumours is not customary.

5.5.3. Regional Metastasis and the Primary Tumour Size (T Staging)

In the initial clinical staging of tongue carcinomas, v.d. Meulen et al. (1983) found that the number of patients with a N+ neck increased with increase of the T stage: T_1 had 13% N+, T_2 38% N+, T_3 53% N+, and T_4 had 65% N+. Bilateral palpable cervical lymph nodes were found in all T stages except in T_1 tumours.

From a study involving patients in our clinic with tongue and floor of the mouth carcinomas (see Table I), it appears that there is no correlation between tumour size and

histologically proven positive cervical lymph node metastases. In the case of T_1, T_2 tumours (less than 4 cm) we found 82%, and in T_3, T_4, tumours (larger than 4 cm) 77% positive lymph nodes in the neck dissection specimens after histological examination.

Table I Histopathological findings

No.	Tumour staging	Negative nodes	Positive nodes/ no capsular involvement	Positive nodes/ with capsular involvement
22	T_1	4	11	7
6	T_2	1	3	2
13	T_3, T_4	3	4	6

5.5.4. Histopathological Aspects of Regional Metastasis.

5.5.4.1. Proven Capsular Invasion of the Nodes and Prognosis

Annyas (1978) found a correlation between a histopathologically proven capsular rupture and prognosis. Verkerk (1978) observed a worse 5-year survival rate in histologically proven capsular rupture in cervical lymph node metastasis. Bartelink (1980) found that postoperative radiation gave a reduced chance of local recurrence in the neck in cases of proven capsular rupture.

Histological findings in neck dissection specimens are therefore of great importance in the prognosis, probably even more than is the clinical N-status. It is known that in clinically attached lymph nodes, 30% have no histologically proven capsule rupture and 6% contain no tumour (Annyas, 1978).

In the series mentioned in 5.5.3., in T_1 and T_2 tumours, 82% positive lymph nodes were observed, of which 39% had penetration of the capsule, and in T_3 and T_4 tumours 77% were positive with 60% penetration. In this small series of patients, capsular penetration appeared to occur more frequently in larger tumours (Table I).

5.5.4.2. Relationship Between Clinical Staging (N Staging) of the Neck and Histopatholgical Evidence of Tumour Tissue in the Lymph Node.

In a study of patients in our clinic with tongue and floor of the mouth carcinomas, after elective radical neck dissections (N_0), we saw 36% histopathologically proven lymph node metastases. This agrees well with the average conversion in the literature (see 5.5.5.). In N_1 stage tumours, we found

45% proven tumour metastasis, and 70% in N_2 stage tumours (Table II).

Table II Histopatholgical findings

No.	N-staging	Negative nodes	Positive nodes no capsular involvement	Positive nodes with capsular involvement.
11	N_0	7	1	3
22	N_1	12	5	5
10	N_2	3	1	6

Finally, it should be noted that a small lymph node is no guarantee for the absence of capsular penetration. Annyas (1978) found that in lymph nodes smaller than 1 cm, there was a 22% incidence of capsular penetration.

5.5.5. Conversion.

We use the term conversion when in a follow-up period an N_0 neck becomes N+. On the basis of conversion percentages, the extent of occult regional metastasis can be determined. In the literature, an average conversion of 33% is given for all T stages of tongue carcinoma (van Andel et al., 1983; Teichgraeber, 1984). V. d. Meulen et al. (1983) found a conversion percentage for tongue carcinoma of 40% for T_1 , 50% for T_2 , 70% for T_3 and 40% for T_4 tumours. The total percentage of regional metastasis is arrived at by summation of the % conversion and the % histopathologically proven positive elective neck dissection specimens.

V.d. Meulen et al. (1983) found for tongue carcinoma 42% for T_1 , 57% for T_2 , 73% for T_3 and 82% for T_4 tumours. These figures are, in our opinion, sufficiently convincing to always opt for elective treatment of the neck in cases of non-superficial growing tongue carcinoma. This is in agreement with the opinion of Steensma (1971) among others, who advise surgery or radiotherapy of the neck in cases of these carcinomas.

5.5.6. The Prognostic Relevance of the Various Clinical Findings in the Cervical Lymph Nodes at the Time of First Admission.

According to Chu and Strawitz (1978) and Koch (1975) there is evidence that the presence of palpable lymph nodes is associated with a worse prognosis. Black and Speer (1960) and Crile (1968) and the AJC (1978) consider that clinically positive lymph nodes classified by the clinician as a metastasis, are a bad prognostic sign.

The degree of fixation of the regional lymph nodes worsens the prognosis according to Fletcher et al. (1963), Votava et al. (1972) and Djalilian et al. (1973).

Rollo et al. (1981) and Annyas (1978) consider that prognosis worsens with the presence of an increasing number of affected regional lymph nodes, and Barkley et al. (1972) and Koch (1974) found in their studies a worse prognosis if contralateral lymph nodes were involved. Langdon et al. (1977) localized metastasis at various levels and found a worse prognosis with more advanced localization of palpable lymph nodes in the direction of cervical lymph drainage.

The multivariate analysis in the investigations of Platz et al. (1982) showed that clinically positive diagnosed lymph nodes indicate a significantly worse prognosis and that clinically positive diagnosed fixed lymph nodes have a worse prognosis than clinically positive lymph nodes which are not fixed. Factors considered to be associated with a worse prognosis, such as the number of affected lymph nodes, contralateral positive lymph nodes and regional distribution in high, middle or low regions of the neck, appeared after univariate analysis in the Dösak study, to give significant differences in prognosis, but not after multivariate analysis.

5.6. Distant Metastasis.

Distant metastasis is considered to be a grave prognostic sign (Probert et al., 1974; Platz et al., 1983).

5.7. Patient Age.

In the younger patient, tumour behaviour is frequently more aggressive (Spiro and Strong, 1971). However, with increasing years, the chance of death from non-tumour related diseases is greater, which influences survival (Lindquist, 1979).

After multivariate analysis, Platz et al., (1983) saw a significant difference between three groups of patients: younger than 50 years, 50 - 70 years and older than 70 years.

6. Surgical treatment of oral cavity carcinoma.

In the 18th century and also later, the surgical treatment of oral cavity tumours was a precarious business. The problems, besides those of anaesthesia and infection, were principally in the area of haemostasis. In those times, there was no question of reconstruction being attempted.

The turning point came at the beginning of this century. The improved surgical and haemostatic techniques, the

developments in general anaestheia and the introduction of antisepsis, resulted in successively better cure rates in the surgical treatment of oral cavity carcinomas. With the acquisition of knowledge of the regional metastasis pattern, Crile (1906) decided to attempt his first radical neck dissection.

The reconstructive possibilities remained limited and the operative mortality rather high. For these reasons, many regarded radiotherapy as the treatment of choice.

In 1940, the real turning point in surgical treatment was reached. The first block resection was attempted in America by Martin (1941), providing the basiis for present surgical techniques. The introduction of the block resection principle had a considerable positive influence on the 3-year survival of oral cavity carcinomas (Verkerk, 1978).

6.1. Surgery of the Primary Tumour.

There is general agreement that in squamous cell carcinoma of the oral cavity, the distance from the resection surface to the tumour must be at least 1.5 cm and that in order to evaluate the tumour-host relationship, scalpel resection is necessary. The limits for curative operability of the primary lesion are determined by the vital structures which in general can not be included in a block resection. Carcinomas with parapharyngeal penetration into the prevertebral fascia, extension to the common carotid, extension into or through the base of the skull, and infiltration into or through the hyoid with crossing of the mid-line or base of the tongue, must be recognized as surgically incurable. Through greatly improved reconstructive possibilities, for example, introduction of myocutaneous island flaps (e.g. Ariyan, 1979) and free microvascular transplantations, the concept of "non-resectable" is now seen in a new light.

6.2. Surgery of Lymph Node Metastasis.

As has already been indicated, there is much less consensus of opinion concerning surgery of the neck. In treatment of the N+ neck, most investigators have preference for surgical treatment (International Conference of Head and Neck Surgery, 1984).

Spiro and Strong (1981) initially performed no elective neck dissection for T_1 , T_2 and T_3 tumours, but on the basis of their moderate results, they now perform elective cervical node dissections for T_2 and T_3 tumours. In contrast, the value of elective neck dissection has been seriously questioned by Tulenko et al. (1966) and Flemming (1970). The functional disturbances after radical neck dissection were for Bocca (1967) a reason to introduce modifications

into the elective surgical process. The discussion within the surgical profession is centered mainly around this area.

There appears to be a growing consensus in favour of obligatory neck dissection for the N+ neck and surgical staging of the neck using a modified neck dissection for the N_0 neck (International Conference of Head and Neck Surgery, 1984).

Surgical staging in the N_0 neck must not be limited to the lymph nodes of the suprahyoidal region (Harrold, 1971); when surgery is opted for, radical neck dissection or modified neck dissection according to Bocca (1967) is always indicated. It appears from the findings of Annyas (1978), that the latter method also has its limitations "in less experienced hands": if removal of the internal jugular vein was omitted, tumour recurrence occurred more frequently in the neck than after a radical cervical lymph node extirpation.

The 2-year survival rate of patients who still showed a positive neck in the follow-up period (conversion), is in the patient material of Teichgraeber (1984), 37% for tongue carcinoma and 32% for carcinoma of the floor of the mouth. V.d. Meulen et al. (1983) arrives at a conversion of:

T_1 , N_0 -> N+ 40%
T_2 , N_0 -> N+ 50%
T_3 , N_0 -> N+ 71%
T_4 , N_0 -> N+ 50% (see 5.5.5.)

These figures concur with those of Spiro and Strong (1971). Thus, surgical staging of the neck may never be omitted. Exceptions to this rule: verrucous carcinoma and the superficially growing T_1 carcinoma, justify expectative treatment of the neck because of their clinico-pathological behaviour.

Suprahyoidal neck dissection appears for the most part to have been abandoned as a mode of treatment for regional metastases (Chu and Strawitz, 1978; Donegan et al., 1982). The suprahyoidal space on the ipsilateral side may be opened only for surgical technical reasons in the surgical approach to the primary tumour and when the tumour crosses the mid-line. If positive lymph nodes are manifest on the ipsilateral side, a modified neck dissection must be carried out in the first instance, or a total radical neck dissection on that side in the second instance.

7. Radiotherapy of oral cavity carcinoma.

7.1. Radiotherapy Versus Surgery.

Because of the only moderate surgical success in the first decades of this century and the development of orthovoltage apparatus (200 to 250 kilovolt tube voltage) in the 1920's,

radiotherapy became the treatment of choice. At that time, with the implantation of radium needles, a combination of interstitial therapy and teletherapy could be utilized in oral cavity carcinomas which were confined to soft tissue (mobile tongue and floor of the mouth).

Radiotherapy is a treatment mode for the smaller oral cavity carcinomas which demonstrate no bone invasion and which in competent hands, results in favourable cure percentages (Frazel, 1971). Evaluation of 5-year survival rates of tongue carcinoma treated primarily by radiotherapy and compared with treatment which is primarily surgical, showed that survival rates for all stages were higher for surgical treatment. Fu et al. (1976) record 5-year survival rates, using primarily radiotherapy of Stage I - 45%, Stage II - 43% and Stage III - 16%. Mendelson et al. (1976) record 5-year survival rates for surgically treated cases of Stage I - 85%, Stage II - 81% and Stage III - 58%. Decroix and Ghossein (1981) who used mainly interstitial therapy together with teletherapy plus surgical dissection of the cervical lymph nodes in cases of positive nodes, record a better 5-year survival rate than Fu et al. (1976), who used only external megavolt radiation.

Panje et al. (1980) conducted a random investigation into floor of the mouth carcinomas, one aspect of which was surgical treatment, the second radiotherapy, and the third aspect pre-operative radiotherapy and surgery. For Stages I and II, the outcomes for all three types of treatment were essentially the same. For Stage III, surgery appeared to be vastly superior to radiotherapy. The 5-year survival rate figures were 25% for radiotherapy (external megavolt therapy) and 75% for radical surgery. It is noteworthy that pre-operative radiation therapy in Stage III floor of the mouth carcinoma did not result in an improved 5-year survival rate in Panje's (1980) study.

For the higher stages of tumours in the tonsillar region and retromalar space, the cure rate of radiotherapy is similarly much less impressive than surgery alone (Weller et al., 1976; Cardinale and Fischer, 1977).

7.2. Interstitial Therapy.

Interstitial therapy (endocurie = brachytherapy) is a treatment for small tongue and floor of the mouth carcinomas which results in a good cure rate (Botstein et al., 1976). The use of interstitial therapy together with teletherapy in tongue tumours results in cures which are comparable with those of surgical treatment (Decroix and Ghossein, 1981). Nevertheless, the popularity of this method is not great in The Netherlands. This was evident in a discussion in 1983 during the Workshop on Squamous Cell Carcinoma of the Tongue and Floor of the Mouth (van Andel et al., 1983). The main

objections raised were that:

1. The treatment must be carried out under general anaesthesia.
2. Tracheostomy is often necessary.
3. Post-treatment effects (pain and ulcers) are of considerable significance.
4. If there is recurrence, it can be recognized only at a late stage because of severe fibrosis and infiltration, so that "salvage surgery" is frequently attempted too late.
5. Radiation necrosis of mandibular bone is described.
6. There is no information concerning such histological parameters as perineural spread or vascular infiltration, nor information concerning staging of invasion.

7.3. Radiotherapy of Cervical Lymph Node Metastasis.

In the 1960's after the introduction of megavolt techniques (Cobalt 60 apparatus), the radiotherapy options for cervical lymph node metastasis were greater. Schneider et al. (1975) and Bataini (1977) found in their patient material, 10% recurrence in N+ necks with lymph node metastases which were 3 cm or smaller. With increase in number and size, or lymph node fixation to the skin and neurovascular bundle, the percentage of recurrence increases. Bartelink (1976) carried out a retrospective study of 143 patients who had undergone only radiotherapy of cervical lymph node metastases. Seventy percent of the primary tumours were staged as T_3 , T_4 . The neck was irradiated with the maximum tumour dose of 7,000 cGy over 7 weeks. Seventy seven percent of the tumours appeared to be incurable. Bartelink also observed a shorter recurrence-free period in well-differentiated carcinomas, in cases with an increase in the number of positive lymph nodes. This confirms the conclusion that radiation of the positive lymph nodes produces an unsatisfactory result in cases of oral carcinoma. The radiotherapeutic cure rate of 81% for lymph nodes of 3 cm or smaller (Schneider et al., 1975) does not concur with this result. The figures in this publication are unbelievably good: the patient group was made up in such a way that in the group of radiotherapeutically treated oral cavity (pharynx and larynx) carcinomas, no recurrence of the primary tumour was confirmed in a 4-year follow-up. A more perfect selection for radiotherapeutic sensitivity of the primary tumour is impossible, and it is therefore possible that the regional metastases were also influenced equally well by the radiotherapy.

7.4. Radiotherapy as Elective Treatment of N_0 Staged Neck.

The most well-known supporters of this treatment are Fletcher and his colleagues (1972). The usual dose is 50 Gy over 5 weeks. The percentage conversion (N_0 -> N+) in Fletcher's patient material (n = 356) drops to 10%. The average

conversion figure in the literature is approximately 33%. Thus, the chance of proliferation of the lymph node metastases after elective radiotherapy is reduced by a factor 3. There are even better results described in the literature (Bagshaw, 1971). V.d. Meulen et al. (1983), in 13 cases of elective radiation of the neck (in which the primary tumour was also radiated), recorded a conversion percentage of 38%, in which the average conversion was equally distributed over all the tumour stages.

7.5. Radiotherapy and Surgery as Combined Treatment.

Combinations of radiotherapy and surgery came into vogue in the 1960's. The motivation for this combination is that surgery in the periphery of the resection boundaries fails if tumour remains in situ, and that radiotherapy in the poorly oxygenated centre of the tumour fails and leaves cells there with the potential for proliferation. By combining radiotherapy and surgery, it was hoped that the advantantages of both could be utilized. The introduction of the radiotherapy/surgery combination has significantly improved local cure (Jesse and Lindberg, 1975; Vikram et al., 1983).

Radiotherapy can be used as pre-operative therapy. It can also be used as primary therapy, saving surgery as salvage therapy where necessary (Fu et al., 1976). A great deal of experience has been gained in pre-operative radiotherapy (Hintz et al., 1979; Strong et al., 1978; Terz et al., 1981). In fact, the prospective randomized trials carried out by these investigators did not reveal any significant improvement in the survival rates for the group which underwent pre-operative radiation treatment in comparison to that which underwent surgery alone.

The advantages of radiotherapy after surgery are particularly clear in the patient group with an increased risk as far as both the primary tumour and the neck are concerned. The survival rate of this group is clearly better with radiation therapy after surgery than without this treatment (Vandenbrouck et al., 1977; Marcus et al., 1979; Snow et al., 1981).

8. Chemotherapy in oral cavity carcinoma.

In the 1970's chemotherapy became an additional treatment method for cases of advanced tumours. Justification was sought in the fact that even in large centres with a reputation in different modes of therapy, the cure percentages for Stage IV oral cavity carcinomas had in recent years improved only slightly or not at all.

Problems in the evaluation of combined treatments which include chemotherapy, are caused by:
1. The different times at which cytostatics can be

administered in combination with other treatment modes, for example:

a. pre-operative (up-front or induction chemotherapy)
b. post-operative (short-duration - in cycles; long duration-maintenance dosage)
c. before radiotherapy
d. during radiotherapy
e. after radiotherapy.

2. The different methods of administration, for example:
   oral
   intravenous pulse
   intravenous continuous
   intravenous intermittent continuous
   intra-arterial pulse
   intra-arterial continuous
   intra-arterial intermittent continuous
   intraperitoneal
   intralesional.

3. Single versus multiple drug therapy

Many "multidrug" chemotherapeutic regimes have been shown to be not significantly better than "monodrug" therapy. The pertinent literature is almost inpenetrable, and the superiority of any combined therapy is difficult or impossible to establish. The great variability in the biological behaviour of oral cavity carcinoma in response to chemotherapeutics, prompts investigation into methods of prediction of tumour behaviour to the chosen therapy which will increase the chance of cure (this thesis).

Although a few cases of regional cure have been described, chemotherapy alone is not an acceptable treatment modality for oral cavity carcinoma. Induction chemotherapy using some combinations (Cisplatinum - 5Fu) has resulted in a high complete response rate when used pre-operatively or pre-radiotherapeutically (Kish et al., 1984). There appears to be a correlation between the percentage of complete response and higher 5-year survival rates (Hong et al., 1979; Weaver et al., 1982).

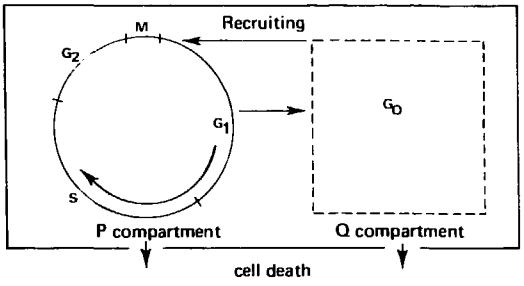
CHAPTER II

Cell Kinetic Parameters of Oral Squamous Cell Carcinoma Determined by Autoradiography, and the Clinical Value of these Determinations.

1. The Cell Cycle.

Proliferating cells regularly traverse a cycle. The incorporation of DNA precursors does not take place during the whole cycle. By means of autoradiography, Howard and Pelc (1951) demonstrated that incorporation of radioactive DNA precursors only occurs during one phase of the cycle (the DNA synthesis or S phase). The time between the S-phase and mitosis is called the post-DNA synthesis gap (G_2-phase). The duration of a cycle (Tc) or intermitotic time, is the sum of the duration of the separate phases (Fig. 1).

Fig. 1



Schematic representation of the cell cycle.

After dividing, a cell can remain in the pool of proliferating cells and divide again after going through the next cell cycle. The cell belongs to the P compartment (proliferating). The cells which do not traverse through a cell cycle are non-proliferating (G_0-phase). The cell then belongs to the pool of resting cells, the Q fraction or the Q compartment (quiescent).
After cell division, the cell can also go into a rest phase (G_0-phase) and then belongs to the Q compartment.
Cells from the G_0-phase can resume proliferation (G_0 -> G_1). This is referred to as recruitment.

2. Cell Kinetic Terminology.

It has been demonstrated (Mendelsohn, 1960), that in many experimental and clinical tumours, not all cells of the tumour are proliferating.
The growth fraction (GF) of a homogeneous cell population is defined as: the number of proliferating cells divided by the number of proliferating and non-proliferating cells.

In order to ascertain the number of cells in the S-phase, one can use autoradiography by determining labelling index (LI) with 3H-thymidine.

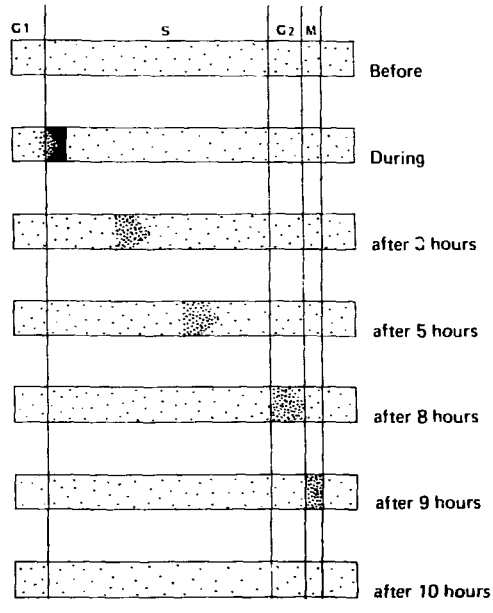
The labelling index (LI) is the number of labelled cells per 100 cells.

The mitotic-index (MI) is the number of mitoses cells per 100 cells.

Bender and Dedrick (1975) defined the phenomenon of recruitment as the amount of cells which are recruited per unit time, from the G_0 (Q compartment) to the proliferative phase (P compartment). Recruitment can be induced by massive tumour cell death, e.g. as a result of radiotherapy or chemotherapy (van Putten, 1974).

Synchronization is the process in which all cells, or a large proportion of cells, traverse cell cycle synchronously (Van Putten et al., 1976) (schematic representation, Fig. 2.).

Fig. 2



Schematic representation of a cell cycle blockade by a chemotherapeutic agent in the early S-phase. Withdrawal of the blockade allows the cells to follow the cycle in cohort. Synchronization has occurred.

After the administration of a phase-specific chemotherapeutic agent (Chapter IV. 1.), and/or ionizing radiation , a temporary cell cycle block can be achieved. The influx from the previous phases then continues during the block. Ideally, the accumulated cells resume cell cycle synchronously.

3.1. Determination of Cell Kinetic Parameters by means of Labelling with Radioactive DNA Precursors (Autoradiography).

Measuring cell kinetic parameters of tumour tissues can be done by labelling the cells with tritiated thymidine either in vivo or in vitro. Cell cycle time and duration of the S-phase can be derived from counting the percentage labelled mitoses (PLM) after labelling with 3H-thymidine in vivo. After an intra-arterial or intravenous pulse injection of the DNA precursor (e.g. 3H-thymydine) multiple sequential biopsies are taken. Histological sections are prepared and coated with a photographic emulsion and stored for some weeks. After developing, fixation and staining of the sections, labelled cells can be counted in the sequential biopsies. In the sequential biopsies PLM values can be determined.

3.2. Cell Kinetic Parameters of Oral Squamous Cell Carcinoma Determined by Autoradiography.

Many authors have determined the cell kinetic parameters of tumours of the oral cavity using autoradiography (Frindel et al., 1968; Tertz et al., 1971; Bresciani et al., 1974; Pape et al., 1975; Helpap et al., 1977; Kreidler, 1976a; Sakuma, 1980). Bresciani et al. (1974) found that the duration of cell cycle (Tc), varied from 52 to 88 hours. The duration of the synthesis phase duration (Ts) varied from 18 to 34 hours. Sakuma (1980) found a Tc between 21.7 and 54.7 hours after applying an in vitro labelling method. Pape et al. (1975) recorded a Tc between 58.4 and 37.2 hours in squamous cell carcinomas of the oral cavity, using a double labelling method with 3H-thymidine and 14C-thymidine. The greatest variability in the duration of the various phases occure in the G_1-phase.

3.3. The Clinical Value of Cell Kinetic Data for Chemotherapeutic and/or Radiotherapeutic Treatment Schedules for Solid Tumours.

Wolberg and Ansfeld (1971) found no correlation between pre-therapeutically determined labelling indices (LI) and the response of solid tumours to chemotherapy with 5Fu. Silvestrini et al. (1984) demonstrated in oral cavity carcinomas, that the drop in LI under the influence of radiation is a measure of the sensitivity of the tumours to radiation. The absolute value of the labelling index, measured before radiation therapy, did not correlate with the effectiveness of therapy in short follow-up. However, if:

$$\frac{\text{LI post-radiation (5f x 200 cGy)}}{\text{LI pre-radiation}} \times 100\% > 70\%$$

a correlation with a prolonged recurrence-free period was

evident. It can be concluded from this that pretreatment cell kinetic parameters have only a limited predictive value with regard to the effectiveness of the therapy, but therapy induced changes in cell kinetic may have prognostic value.

Helpap et al. (1977) were able to determine the cell kinetic parameters by means of double labelling with 3H-thymidine and 14C-thymidine in 9 of the 33 patients with an oral cavity carcinoma. The applied combination therapy which followed was made dependent on the recorded cell kinetic data. The duration of the intravenous 5Fu administration was adjusted according to the calculated TG_1 (G_1-phase time). Radiation with 150 cGy then followed, after the calculated Ts (DNA synthesis-phase time). The length of the S-phase varied from 7.3 - 17.9 hours, the Tc from 20 - 120 hours. All tumours had relatively high labelling indices. These investigators noticed a rapid (within 4 weeks) and complete response of the carcinomas in these cases of combination therapy with cell kinetically adjusted fractionation intervals and in the follow-up period it was evident that one third of the patients were recurrence-free. Two thirds of the patients however, died, (half with an early recurrence and the other half with a late recurrence).

To summarize, it can be concluded that the variation in duration of cell cycles necessitates knowledge of the individual cell cycle parameters in order to enable optimal treatment mode. The determination of the individual cell kinetic parameters by double labelling in vitro appeared to be useful in only one third of cases (Helpap et al., 1977). This method cannot, therefore, be accepted as clinically applicable.

CHAPTER III

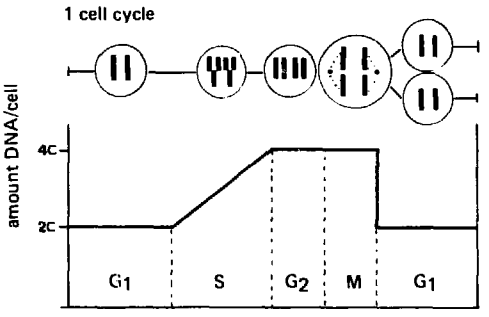
DNA Flowcytometry

1. Introduction
2. Investigations of (Partial) Synchronization in Solid Tumours Using Flow Cytometry.
3. The Clinical Application of Flow Cytometry (general)
4. The Clinical Value of Flow Cytometric Determinations During Treatment of Oral Squamous Cell Carcinoma.

1. Introduction.

The relative quantity of DNA per cell is determined by DNA flow cytometry. The DNA content per cell increases through cell cycle (Fig. 3). In G_1 phase, a di- or euploid quantity is measured (2C). The term C (content) is used in measurements of the DNA level, this being the haploid DNA quantity of a mammalian cell. 2C coincides with the di- or euploid quantity. 4C is therefore the tetraploid quantity, etc. When tumour cells contain more than the diploid amount of DNA after mitosis, then the cells have an aneuploid DNA content.

Fig. 3



The amount of DNA of individual cells during passage through the different phases of the cell cycle. DNA content of the individual cells during the cell cycle progression.

In the S-phase, DNA content is reduplicated. In the G_2+M-phases, a doubled quantity of DNA is encountered. When the DNA level of a large number of cells from a tumour biopsy is determined by means of flow cytometry, a histogram of the relative DNA distribution in that tumour biopsy is obtained. From the histogram, the percentage of cells in the G_1+ G_0-phase, the S and the G_2+M-phases can be calculated. In the case of a diploid tumour cell population, the diploid normal cells and the tumour cells cannot be recognized separately by this method. In an aneuploid cell population (Figs 4. and 5) the G_1 tumour cell population is not contaminated by the normal cells.

The distribution of cells over the cell cycle gives an impression of the proliferative activity of a tumour (Tannock, 1978; Colly, 1980 and Rutgers, 1985).

The ploidy of a tumour is expressed by the DNA index. The DNA index is the quantity of DNA in the G_1 phase of the tumour cell population divided by the quantity of the DNA in the G_1 phase of the normal host cells.

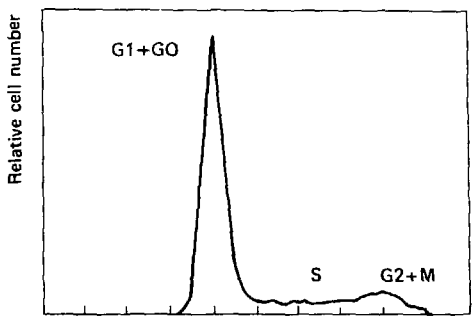


Fig. 4

Histogram of a diploid tumour, the G_1 and G_0 cells of the tumour and the normal cells are registered in the same peak.

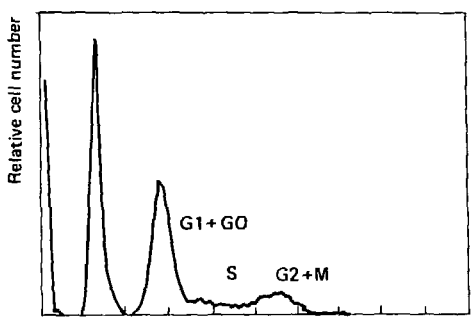


Fig. 5

Histogram of ananeuploid tumour; the (diploid) cells are registered in the first in the G_1 and G_0 phase high peak. The tumour cells produce a separate recognizable peak.

2. Investigations of (Partial) Synchronization in Solid Tumours Using Flow Cytometry.

Synchronization appears to occur in many tumour models and cell lines after chemotherapy or radiotherapy. The extent of the growth fraction partly determines the extent of synchronization. Göhde and Barlogie (1974) demonstrated, in an exponentially growing Ehrlich ascites tumour, that all ascites cells were to be found in the G_2+M-phase (100%) six hours after intra-peritoneal administration of 1.25 mg/kg Adriamycin. The increase of cells in the G_2+M-phase was significantly less (13%) in solid tumours of the same cell line.

Schumann and Göhde (1979) were also able to determine an increase of cells in the G_2 +M-phase in a solid Ehrlich ascites tumour by means of flow cytometry. This increase did not differ significantly with different dosages of Bleomycin in time (with equal total dosage).

Synchronization of solid tumours also occurs with irradiation. Schumann and Göhde (1974) showed, by means of flow cytometry, that an almost complete G_2+M accummulation occurs within 18 hours in an Ehrlich ascites tumour in vivo. Göhde (1973) proved that the duration of this accumulation is dose dependant. Zywietz and Jung (1980) demonstrated a reversible block at the beginning of the S-phase and G_2+M-phase after radiation of the solid Walker tumours. The percentage G_2 +M cells increased after 10 hours from 10.6 to 36.4. In two other tumours, the changes in the distribution over the different phases of the cycle were also maximal after 10 hours.

3. The clinical Application of Flow Cytometry (general).

It is possible, with flow cytometry, to determine the following parameters:
a. ploidy, i.e. relative DNA content.
b. distribution of cells within the cell cycle.

ad.a. Müller et al. (1981, 1983) and Ensley (1985) have not yet been able to derive any prognostic significance from the differences in the relative DNA content of the oral squamous cell carcinomas examined. In cervical and mammary tumours, Rutgers (1985) was able to find, together with other prognostically relevant factors, a correlation between the ploidy and the clinical behaviour of the tumour.

ad.b. Attempts to monitor cancer treatment by studying altered cell cycle distribution have not always been successful (Tannock, 1978), although accumulation of cells in certain cell cycle phases correlates with clinical response during chemotherapy in treatment of some leukaemic diseases (Colly, 1980).

4. The Clinical Value of Flow Cytometric Determinations During Treatment of Oral Squamous Cell Carcinoma.

Wannemacher et al.(1974) and Esser and Wannenmacher (1979) investigated the accumulation of cells in the G_2+M-phase of the cell cycle in non-resectable oral squamous cell carcinomas by means of flow cytometry before and after administration of 5 Fluorouracil, and in other patients before and after treatment with Bleomycin. After administration of 5Fu intravenously continuously during a period of 12 hours, only one third of the patients showed a significant accumulation 10 to 14 hours after termination of the infusion. The mean increase in the percentage cells in the G_2+M-phase for the whole patient group increased on average by a factor of 1.2. Two groups of patients were treated with Bleomycin. The percentage of G_2 +M cells increased by a factor of 2.

Although the accumulation in the G +M-phase after Bleomycin administration appears to be greater than after 5Fu administration, the combination treatment of radiotherapy with Bleomycin did not show significant advantage over radiotherapy with 5Fu, when evaluated clinically.

Kreidler (1976a.) and Barlogie et al. (1982) have shown that changes in the distribution of cells over the cell cycle caused by therapy, are related to the proliferative activity (GF). According to these investigations, the extent of change in the fraction of cells in the different phases of the cell cycle is a prognostic factor in the effectiveness of the therapy, and can be used as a biologically relevant cancer classification for chemotherapy and/or radiotherapy.

To summarize:

1. In comparison with autoradiographic methods,(CH.II), flow cytometry is simple and less time consuming and many more cells can be counted than with autoradiography.
2. Flow cytometric monitoring during therapy can give insight into the change of cell kinetic parameters after administration of the first doses of radio and/or chemotherapy, and may provide an objective biologically relevant prognostic parameter.

CHAPTER IV

The Influence of Chemotherapy and Radiotheraphy on Cell Cycle Kinetics and the Usefulness in Monitoring Cell Cycle Perturbations in Attaining Better Therapy Schedules.

1. The Influence of Chemotherapy on Cell Cycle Kinetics.
2. The Influence of Radiotherapy on Cell Cycle Kinetics.

1. The Influence of Chemotherapy on Cell Cycle Kinetics.

Drugs have been classified according to their effectiveness in cell killing activity in relation to the kinetic status of the exposed cells.

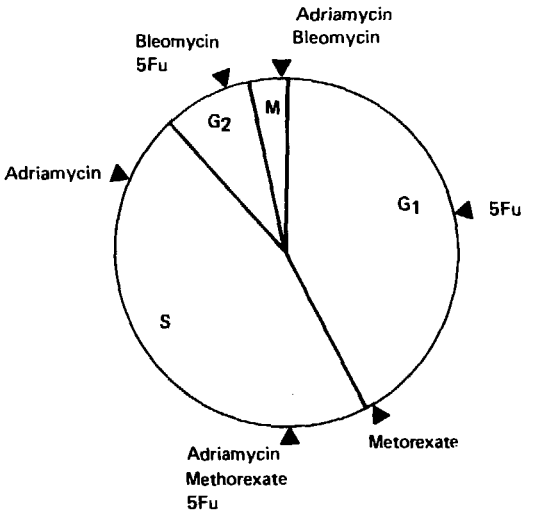
The classification of Skipper (1971) is as follows:
a. Cell Cycle Stage Specific (CCSS) drug: kills cells only if they are exposed in a sensitive phase of the cell cycle.
b. Cell Cycle Stage Non-specific (CCSN) drug: kills cells in all phases of the cell cycle to an almost equal extent.

Bruce et al. (1966) classified as follows:
a. Phase specific drug: kills cells only if they are exposed in one or more sensitive phases of the cell cycle.
b. Cycle specific drug: kills cells in all phases of the cell cycle to an almost equal extent.
c. Cycle non-specific drug: kills cells in all phases of the cell cycle and also cells of the Q compartment (resting cells).

Cell cycle phase specificity therefore means that cells in a certain phase are more sensitive to the administered cytostatic drug than in other phases (Hill and Baserga, 1975). Cells in a sensitive phase were either killed (cytotoxic effect) or delayed in their progress through the cell cycle (cytostatic effect), depending on the concentration of the cytostatic drug (Barranco and Humphrey, 1976; Terasima 1976a; Barlogie et al., 1976). Synchronization can be achieved by the use of phase-specific drugs. Cytostasis effects a blockage in cell cycle progression in the sensitive phase, by which accumulation occurs through the influx of cells from the previous phase(s) during the blockage (van Putten, 1976; Barlogie et al., 1976; Göhde et al., 1979). If cells thereafter resume their cycle, progress synchronously and arrive synchronously in a following phase, then the cells can be killed by a cytostatic agent specific for that cycle phase (Fig. 2.) (Hill and Baserga, 1975).

For many chemotherapeutic drugs, the cell cycle phase specificity is known from in vitro investigations of synchronized mammalian cell lines (Fig. 6.). Knowledge of the specific effect on the cell cycle phases was used in the seventies (van Putten, 1974; Hill and Baserga, 1975) in order to compile complicated chemotherapeutic schemes to fully exploit synchronization and recruitment. Great therapeutic expectations were attached to these schemes (Schabel, 1969; Hill and Baserga, 1975; Constanzi et al., 1976; Braunschweiger et al., 1981). In practice, they could not be realized (Esser and Wannenmacher, 1979; Tannock, 1978). The fact that synchronization cannot be fully exploited in solid tumours is partly a result of the following factors:

Fig. 6



Sites where the named cytostatic agents produce blockade or inhibition of cell cycle progression.

a. The vascularization of solid tumours decreases, in general, from the periphery to the centre (Tannock, 1968, 1972; Pavelic et al., 1981). The vascular architecture of a solid tumour therefore ensures that there are different concentrations of the chemotherapeutic agent at the centre and at the periphery of the tumour (Tannock, 1968). For this reason, in the central part of a solid tumour, synchronization with chemotherapeutic drugs will not occur or will be less rapid.

b. The growth fraction in solid tumours is, in general, greater at the periphery than at the centre (Bennington, 1969; Peckham and Steel, 1972; Kreidler, 1976).

c. Poor capillary perfusion, tending to capillary stasis, and shunt perfusion may cause an asynchronous withdrawal of the effect of a chemotherapeutic block.

d. Chemotherapeutics are often cytotoxic in one phase, and cytostatic in another phase, at the same concentration. As a result of this phenomenon those tumour cells in the "cytostatic" phase are protected against cell death (Barranco and Humphrey, 1971). A higher dose of Bleomycin, for example, is lethal for cells in the M-phase; the block in the G_2-phase will, indeed, bring about synchronization, but will also protect the arrested cells against cell death. When the

cohort reaches the M -phase, the concentration of the Bleomycin has become too low to cause cell death (Barranco and Humphrey, 1971).

e. The timing of synchronization will be different in different tumours. Therefore, it is not possible to apply general fractionation schemes and simultaneously exploit the therapeutic gain from induced synchronization.

2. The Influence of Radiotherapy on Cell Cycle Kinetics.

A blockage in cell cycle progression occurs in the most radio-sensitive phases (G_1 -S transition, G_2 -phase and mitosis). Sinclair and Morton (1966) demonstrated that cells in the G_2-phase are the most sensitive to radiotherapy. The duration of the blockage is dose-dependent and is also dependent on the radio-sensitivity of the tumour cells (Mitchel et al., 1979).

Synchronization will occur in the same way as described for the chemotherapeutic influence of phase-specific agents on the cell cycle (Ch. IV, 1). The maximal radiation-induced accumulation of cells in the G_2-phase will depend on the number of cells in the P compartment (Kal, 1973). The time necessary for maximal accumulation in the G_2-phase is:
T max acc. = Tc - TG_2 + Tx (where Tx is the sum of the blockage duration in the other cell cycle phases).

Todoroki et al. (1982) found enhanced radiation lethality in partially synchronized solid murine tumours. Studies by Braunschweiger et al. (1979), with solid transplantable murine mammary tumours, in which different radiotherapeutic doses and different sequences in time were applied, demonstrated that the most effective schedules for local control were those in which a second radiation dose was administered at the end of the "recovery phase". He defines the "recovery phase" as the period in which, after radiation, the Tc is almost normalized and the progression of the cells from the G_1 to S and from the S to G_2-phases is normalized. At the end of the "recovery phase", a maximum accumulation of the proliferating tumour cells in the G_2-phase is effected, so that a second dosage of radiotherapy will destroy almost all synchronized cells. This will result in an optimal radiation effect.

To summarize, Braunschweiger et al. (1979) has demonstrated that optimization of the interval between the radiation fractions is possible if the time of the recovery phase can be determined. An individualized treatment schedule with chemotherapy and/or radiotherapy based on the prediction of changes in cell cycle distribution by each treatment modality during the course of therapy, is not possible due to the many

different and interfering cell cycle effects. This in turn leads to corresponding difficulties in the interpretation of the effects, but however flow cytometry can provide indications for the occurrence of cell kinetic changes and repopulation processes.

The aim of the present research is to investigate the existence of a correlation between changes (caused by the therapy) in the distribution of cells over the cell cycle and the chance of sterilization of the tumour.

CHAPTER V

I-BLEOMYCIN DATA

II-ADRIAMYCIN (DOXORUBICIN) DATA

I. BLEOMYCIN DATA

1. Introduction
2. Cytotoxicity and Cell Kinetics
3. Pharmacokinetics
4. Clinical Toxicity

II. ADRIAMYCIN (DOXORUBICIN) DATA

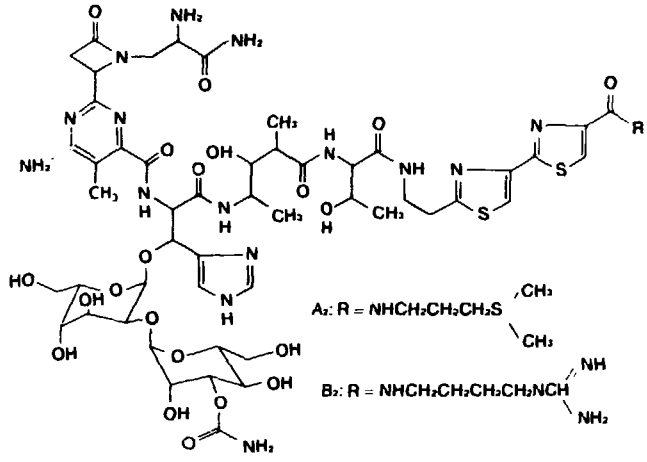
1. Introduction
2. Cytotoxicity and Cell Kinetics
3. Pharmacokinetics
4. Clinical Toxicity

I. BLEOMYCIN DATA

1. Introduction.

Bleomycin is a group of complex glycopeptides produced by Streptomyces verticillus and is obtained through various consecutive separation procedures. Bleomycin does not itself possess cytotoxic activity; only when the free carboxyl group forms a peptide with an amine or polyamine does cytotoxicity occur. There are more than 200 natural Bleomycins known to be the product of Streptomyces verticillus. Commercial Bleomycin is predominantly a mixture of Bleomycin A-2 and Bleomycin B-2 (Fig. 7).

Fig. 7



Structural formula of Bleomycin.

The mechanism of action of Bleomycin is probably related to intercalation in DNA between base pairs, in which the predisposition sites are thymidine and adenosine bases (Müller and Zahn, 1976; Miyaki and Ono, 1976). The efflux of thymidine bases leads to a break in the helix (single and double-strand breaks). At high concentrations of Bleomycin, all four nucleotide bases are split off from the DNA but at low concentrations an apparent preference exists for the thymidine location.

Iqbal et al. (1976) demonstrated, with a DNA elution method, that after X-ray treatment the DNA breaks are distributed randomly over the helix, and that Bleomycin has this effect on predisposition sites.

2. Cytotoxicity and Cell Kinetics.

The dose-survival curves of mammalian cell lines shows, in general, a biphasic curve with a smaller D_0 for low concentrations and a greater D_0 for the higher concentrations (see as example Fig. 13, Chapter VI)*. It is evident, from the various publications, that Bleomycin has a preference for the G_2 -M localization in the cell cycle and to a lesser degree for the G_1-S transition (Barranco and Humphrey, 1976; Bhuyan and Fraser, 1974; Nagatsu et al., 1972; Terasima et al., 1976a,b.; Tobey, 1972; Watanabe et al., 1974). Synchronized populations incubated with Bleomycin are inhibited in the M-phase, resulting in an accumulation in the G_2 -phase (Barranco and Humphrey, 1976; Terasima et al., 1976 a,b.). There are however, enough reasons to assume that Bleomycin is not a purely cell cycle specific or phase specific drug. Barranco and Humphrey (1976), Hahn et al. (1974) and Terasima et al. (1976 a,b) demonstrated that the sensitivity of cells in the plateau phase was greater than in the log phase.**

Non-dividing cells are most sensitive to the cytotoxic effects of Bleomycin. Olah et al. (1978) found in a C.H.O. cell line, a sensitivity to Bleomycin which was 18 times greater in a culture at the plateau phase than a similar cell culture at the log phase. Cultures at both the plateau and the log phases can recover from PLD (potentially lethal damage). Log phase cells are, however, more efficient in this regard (Barranco and Humphrey, 1976; Terasima et al., 1976 a,b.). Recovery from PLD is inhibited by repeated incubation with Bleomycin.

Incubation time - survival curves (at constant concentration) also results in a biphasic curve with an upward concavity. After a certain time the curve begins to flatten out. This means that the cells become less sensitive to Bleomycin after a certain incubation period. This relative resistance to Bleomycin in the cell occurs within a few hours. From research performed by Urano et al. (1973) and Terasima et al.(1976 a,b.) it is apparent that multiple low doses bring about a greater growth inhibition than do high single doses.

3. Pharmacokinetics.

After intravenous bolus injection, Bleomycin disappears biphasically in the serum at an average initial half life of

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* The D_0 is a measure of the slope of the dose - survival curve in the exponential part of the curve, and is the amount of chemotherapeutic agent or radiotherapy necessary to reduce the survival fraction by e^{-1}

** A culture in the log phase grows exponentially (all cells are in the P compartment). In a culture in the plateau phase, as many cells die as are produced by cell division in unit time.

24 minutes and an average terminal half life of 4 hours (Alberts et al., 1979). The half lives following termination of a continuous infusion are also biphasic but longer than those observed following intravenous bolus administration (79 minutes and 9 hours respectively). Approximately 50% of the dose is excreted renally in 24 hours (Alberts et al., 1979). Adjustment of the dosage in cases of renal function disorder is therefore necessary. Many investigators have recommended the use of a continuous infusion on the basis of the relatively short half life of Bleomycin (Krakoff et al., 1977).

A steady state serum concentration of 146 ng/ml could be achieved in most patients within 24 hours of the start of infusion (Broughton et al., 1977).

4. Clinical Toxicity.

Pulmonary toxicity is one of the major complications of Bleomycin therapy and may be progressive and irreversible, leading in some cases to respiratory failure and death.

The incidence of this toxicity varies but is probably 5 - 10% with a mortality rate of about 10%. Patients at risk are older than 70 years and have received a total cumulative dose greater than 400 mg (Blum et al., 1973; Weiss and Muggia, 1980; Collins, 1980).

The clinical presentation of Bleomycin-induced pulmonary toxicity is non-specific. The symptoms develop insidiously and occur between 4 and 10 weeks after initiation of the therapy. According to Haas et al. (1976), the route of administration plays an important role in inducing pulmonary toxicity (23% incidence after intravenous bolus compared to 15% following intramuscular administration). When Bleomycin is administered prior to general anaesthesia and surgery, care must be taken to minimize the concentration of inspired oxygen since the risk of developing pulmonary fibrosis is considerable (Goldiner et al., 1978).

Minor toxic complications of Bleomycin, are gastro-intestinal side effects such as nausea, vomiting and anorexia, which are generally mild in nature. Mucocutaneous symptoms are the most common manifestation of toxicity, probably due to the reduced occurrence of Bleomycin hydrolase in skin and mucous membranes (Müller and Zahn, 1976). Skin reactions occur within 2 - 4 weeks after initiation of therapy, and the severity depends on the cumulative dose and the individual sensitivity. Skin toxicity is more likely to be elicited by exposure to prolonged low concentrations than to transient high peak concentrations (Haas et al., 1976).

Oral mucosal ulcerations, alopecia, hyperpigmentation of pressure areas are usually reversible and gradually disappear after Bleomycin treatment is stopped.

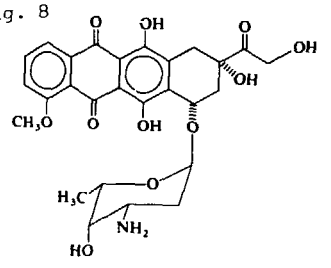
Drug induced fever is common, and more severe during the first doses. It occurs in 40 - 60% of patients within 2 - 6 hours after the beginning of the therapy.

II. ADRIAMYCIN (DOXORUBICIN) DATA

1. Introduction.

Adriamycin (doxorubicin) is an anthracycline antibiotic which was originally obtained from fermentation of the Streptomyces peucetius var. caesius. Purification of Adriamycin occurs through chromatographic extraction (Arcamone, 1981) (for structural formula, see Fig. 8.). As with Bleomycin, the biological activity of Adriamycin is caused by the specific linkage with the DNA by intercalation between two complementary bases (Di Marco, 1971). This results in strand breaks in the DNA helix whereby the DNA replication is delayed (Momparler et al., 1976; Zunio et al., 1975). From the research conducted by Byfield et al.(1977), it is apparent that breaks in the DNA helix (strand breaks) are not repaired.

Fig. 8



Structural formula of Adriamycin.

Noël (1981) has established that a steady state condition can be achieved in a fibroblast cell line after 6 to 10 hours' incubation with Adriamycin. At the end of the incubation period, 40% of the accumulated Adriamycin is to be found in the nucleus and 20% in the lysosomes. The intercalation of Adriamycin in the DNA helix is the principle cause of the development of an intracellular concentration gradient. There is no indication of active transport of Adriamycin over the cell membrane.

2. Cytotoxicity and Cell Kinetics.

The dose-survival curves of mammalian cell lines exposed to

Adriamycin are biphasic (the same as Bleomycin), with a smaller D_0 for the low concentrations and a greater D_0 for the higher concentrations (Barranco et al., 1973). Barranco explains the reduced sensitivity of part of the cell line by genetic non-homogeneity of the CHO cell line used. Survival fractions of synchronized cells show that both the mitosis and the early S-phases are the most sensitive to Adriamycin. Kim and Kim (1972) also found the early S-phase of HeLa cells to be the most sensitive. From research by Kim and Kim (1972) and Barranco and Novak (1974), it appears that the greatest effect is achieved by doses between 3 and 5 microgr/ml during 1 hour incubation. Wang et al. (1972) demonstrated that high concentrations (0.5 microgr/ml) caused an irreversible blockade (cytotoxic effect = progression stop) in the S-phase and that with low dosages (0.01 microgr/ml), a reversible blockade occurred (cytotoxic effect = progression delay).

The fact that Adriamycin must not be seen as a typically phase-specific drug is shown by the research of Barranco and Novak (1974) which demonstrated that cultures in the plateau phase were also sensitive to Adriamycin. Ritch et al. (1982) found that the lethal effect is dose dependent in cell lines in both the log phase and the plateau phase. Concurring with Barranco and Novak (1974), Ritch et al. (1982) found that the sensitivity is greater for log phase cultures (for every concentration examined) than for cultures in the plateau phase.

3. Pharmacokinetics.

Benjamin et al. (1973) found a biphasic decrease in plasma concentration of Adriamycin in tumour patients, in which the short half life was 1.1 hours and the long half life was 16.7 hours. Alberts et al. (1981) found a half life of 7 minutes for first exponential component, and of 31 hours for the second exponential component.

Bachur et al. (1981), using a radio-immunoassay (RIA) procedure, was able to measure a three-phase plasma disappearance curve for Adriamycin. The initial phase had a short half life of 11 minutes, then a second intermediate phase occurred with a half life of approximately 3 hours, followed by a terminal phase with a long half life of about 27 hours. All of these plasma half lives were determined after single bolus administration. The liver clearance of Adriamycin is 60% after a single passage through the liver.

Adriamycin is excreted mainly in bile. In icteric patients and patients with extrahepatic biliary obstruction, the dose rate should be appropriately adjusted.

4. Clinical Toxicity.

The toxic effects induced by Adriamycin are dose related. One of the most dramatic toxicities is myelosuppression, which occurs in 60 - 80% of patients. Leucopenia is therefore the most predominant haematologic toxicity, and its severity is dose dependent and related to the regenerative capacity of the bone marrow. The leukopenia generally occurs during the second week of therapy and returns to normal in the second to fourth week after therapy has been completed. Thrombocytopenia and anaemia are usually not a significant problem, but they occur due to the same myelosuppression.
Another major toxic effect is cardiotoxicity which is dose dependent. Electrocardiographic changes, which are usually reversible, are reported in 6 - 30% of patients (le Frak et al., 1973). Drug induced cardiomyopathy produces significant morbidity and mortality and there is no correlation with pre-existing heart disease. In cases of rapid progressive heart failure with cardio-respiratory decompensation, the interval between the last Adriamycin administration and the onset of heart failure is 1 - 6 months; there is a clear correlation with the total dose, and with patient's risk of congestive heart failure. Patients with a total dose above 500 mg/m^2 are therefore predisposed (Cortes et al., 1973; Carter, 1975; Benjamin, 1981).

Other toxic effects are:

a. drug induced stomatitis, erythema and ulcerations of the oral mucosa.
b. alopecia of the scalp, axillary and pubic areas in nearly 100% of patients. This is usually reversible in a few months.
c. Gastro-intestinal toxicity, such as vomiting, occurs in a minority of cases.

Extravasation during administration produces severe local tissue necrosis.

CHAPTER VI

Theoretical Aspects of Combination Treatment

1. Nomenclature of Combination Treatment
1.1. Sensitization.
1.2. Supra-additivity.
2. Mechanisms which can be Utilized in Combination Treatment.
2.1. The Respective Independence of the Toxicity of Radiotherapy and Chemotherapy for Normal Tissues (also referred to as Anatomical Resistance).
2.2. The Respective Independence of Systemic Toxicity of Different Chemotherapeutics (also referred to as Toxicity Independence).
3. Recovery of Cell Kinetic Parameters after Administration of Chemotherapy and Radiotherapy.
4. The Determination of an Optimal Sequence in Combination Treatment with Radiotherapy and Chemotherapy, using in vitro Techniques.
5. Experimental Investigations of Combination Treatment with Bleomycin and Radiotherapy.
6. Experimental Investigations of Combination Treatment with Adriamycin and Radiotherapy.
7. Experimental Flow Cytometric Investigations after Combined Administration of Chemotherapy and Radiotherapy.

1. Nomenclature of Combination Treatment.

In the literature, many terms are used to refer to the studied effects of combined radiotherapy and chemotherapy on the tumour. In order to reach some uniformity in the discussion and evaluation of combination therapy, a definition of the various terms will be attempted. When authors are cited, the concepts will be discussed according to the definitions below, even if other names for the mentioned phenomena are used in the authors' publications.

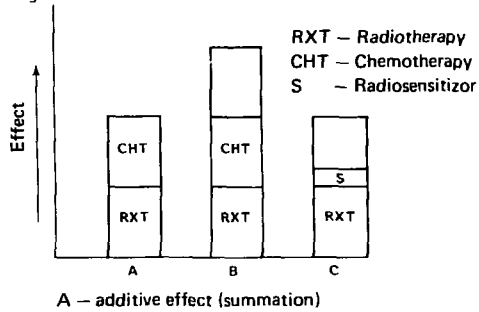
An improved therapeutic effect on the tumour is a general term and a combination of the following concepts:

1.1. Sensitization (Fig. 9c).

This term is used for the phenomenon in which a substance itself has little or no effect on a tumour but has, in combination with another therapeutic agent, a greater effect on a tumour than can be predicted from a dose-effect curve of the therapeutic agent used. The sensitization is then effected by the "inactive substance", e.g., increased oxygen partial pressure in radiation therapy; Misonidazole in radiation therapy.

1.2. Supra-additivity (synonyms: potentiation, synergism) (Fig. 9).

Fig. 9



A – additive effect (summation)
B – supra-additive effect
C – radiosensitization effect

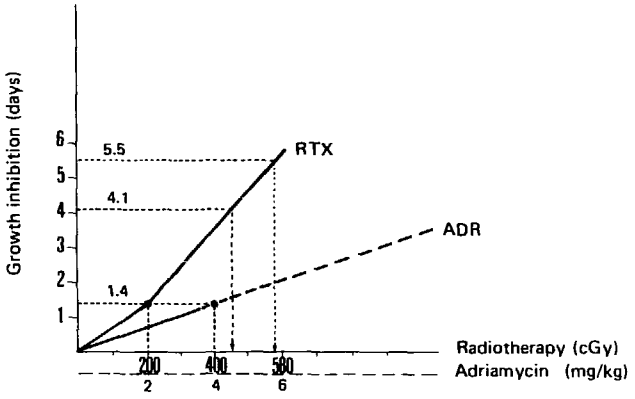
Radiation-chemotherapy interaction

The term supra-additivity is used by Steel and Peckham (1979a.) when the effect of combined therapy is greater than can be expected on the grounds of the analysis of the dose-effect curves of each treatment mode separately. Steel and Peckham (1979a.) introduced the isobologram (Fig. 11) for the interaction between two treatment modes, whereby one can speak of a supra-additive effect below the addition-envelope

(A), of additivity between the two curves (B), and of a sub-additive effect above the curve (C).

An isobologram as in Fig. 11 is obtained when the separate dose-effect curves for chemotherapy and radiotherapy are set out graphically (Fig. 10). The calculation of the separate

Fig. 10



Dose-effect curves of Adriamycin and radiotherapy

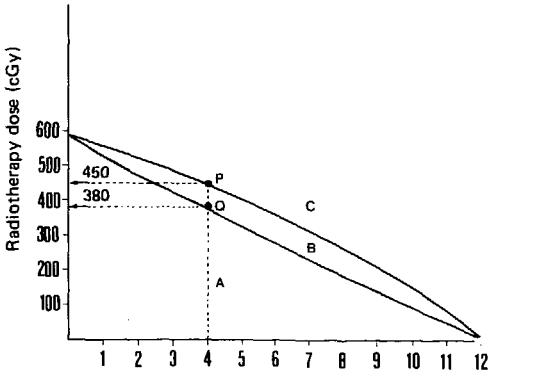
highest and lowest points (points P and Q) for every dosage of a chemotherapeutic agent is then possible (text Figs. 10 and 11).

When combined therapy results in a particular iso-effect on the tumour which is evident below the addition envelope, we then speak of a supra-additive effect (Fig. 11:A). When the iso-effect point lies in the lowest part of the addition-envelope (Fig. 11:B), various effects other than purely supra-additive mechanisms at the macromolecular level in the tumour cell can be responsible for the recorded increased effect on the tumour. In in vivo animal studies with a solid tumour, this effect can be due to, among other things:

a. The positive effect of synchronization.
b. The occurrence of recruitment.
c. Decrease in the size of the tumour.

Clinically, it is extremely difficult to distinguish between additive, supra-additive and sub-additive effects. In vitro and in vivo experiments can however form the scientific basis for a clinical/therapeutic model, whereby a combination treatment can possibly lead to a better therapeutic result.

Fig. 11



A – supra-additive
B – additive

The lowest and highest point of an isobologram of a given dose (Adriamycin) can thus be calculated from the dose effect curves of Adriamycin and radiotherapy. For instance, a dose of 4 mg/kg Adriamycin causes a growth inhibition of 1.4 days corresponding with 200 cGy (Fig. 10). When a combined effect of, for instance, 5.5 days growth inhibition is necessary with a given dose of 4 mg/kg, 4.1 days (5.5 - 1.4 days) inhibition is induced using radiotherapy. This corresponds with 450 cGy (Fig. 10). This is the highest point of the isobologram (point P).

5.5 days growth inhibition corresponds with 580 cGy. When the effect of 4 mg/kg Adriamycin is subtracted the lowest point of the isobologram is obtained (point Q, 580 - 200 = 380). This calculation can be made for all dosages thus creating an addition envelope.

2. Mechanisms which can be Utilized in Combination Treatment.

The advantage of combination chemotherapy and radiotherapy goes even further than the desired (supra)-additive effect on the primary tumour if the following mechanisms can also be exploited.

2.1. The Respective Independence of the Toxicity of Radiotherapy and Chemotherapy for Normal Tissues (also referred to as Anatomic Independence (Kreidler, 1976a.)).

The treatment combination which does not exceed the maximum tolerable systemic and local toxicity and produces the greatest effect on the tumour, is in general the treatment indicated. Increased radiation damage can occur in vital organs in combined chemotherapy and radiotherapy.

Combination treatment can raise the lethality to such an extent that the possible positive therapeutic effect on the tumour is no longer clinically relevant. Increased radiation damage to lung tissue has been shown in combination with Adriamycin and Bleomycin (Phillips and Fu, 1976; Steel et al., 1979b). Dethlefsen and Riley (1979b) and Burholt et al. (1977) demonstrated a higher toxicity for intestinal epithelium in mice after a combined treatment with Adriamycin and radiotherapy, than after radiotherapy alone.

The fact that in oral cavity tumours, the irradiated area can be limited and does not encroach on vital organs, is a great advantage which supports the use of combined chemotherapy and radiotherapy. With chemotherapy the anatomic independence can be maximally utilized when the intra-arterial infusion is strictly localized to the tumour region (Bilder, 1968; Kreidler, 1976).

2.2. The Respective Independence of Systemic Toxicity of Different Chemotherapeutics (also referred to as Toxicity Independence (Steel and Peckham, 1979a)).

We speak of toxicity independence when two partially effective anti-tumour drugs, which cannot individually cause high response rates, as they would exceed the toxic limits, can lead to high response rates when used together, without exceeding the toxic limits. In other words, the maximal dosage of both treatment modes does not have to be greatly decreased as the toxicity for vital organ systems is not additive. When the sum of the toxicity increases less than the additional effect of the combination treatment on the tumour, the therapeutic result is greater.

The combination of Bleomycin and Adriamycin offers the advantage of toxicity independence and can thus result in a clinically synergistic effect (Carter, 1976).

In vitro studies have, in fact, demonstrated that bone marrow stem cells are relatively insensitive to high concentrations of Bleomycin, while there is an obvious toxic effect on bone marrow stem cells when Adriamycin is administered (Ch. V, 1.3.). Dose-dependent lung damage occurs with Bleomycin and is absent with Adriamycin treatment. Dose-dependent cardiotoxicity occurs with Adriamycin, but not with Bleomycin (Ch. V, 2.3.).

3. Recovery of Cell Kinetic Parameters after Administration of Chemotherapy and Radiotherapy.

Recovery of cell kinetic parameters after administration of radiotherapy or chemotherapy means that all fluctuations at the biochemical macromolecular level in the cell and changes

in cytotoxicity which arise after chemotherapy and/or radiotherapy, have reverted to their original state or have attained a new state of balance.

Much knowledge concerning tumour cell behaviour during combination treatment has been acquired through in vivo and in vitro determinations of cell kinetic changes. The interpretation of the results obtained often raises problems, due to the diversity in design of the experiments, partly because the correlation between the cell kinetic changes and the therapeutic effect is not always simple (Esser and Wannenmacher, 1979). In applying the experimental in vivo and in vitro research data to the clinical situation, the fact that most experimental model tumours used have a large growth fraction, in contrast to the human oral cavity carcinoma, also has to be taken into consideration (Bresciani et al., 1974). The recording of cell kinetic changes during combination treatment of known transplantable tumours or cell cultures provides, however, the only possibility of investigating the value of a combination treatment.

4. The Determination of an Optimal Sequence in Combination Treatment with Radiotherapy and Chemothrapy, using in vitro Techniques.

By means of a single administration of a chemotherapeutic agent followed by radiotherapy (or vice versa), with a varying time interval, the effect of each of the time intervals can be determined. In the ideal situation, a curve as depicted in Figure 12 can be drawn, from which the optimal sequence of treatment can be read.

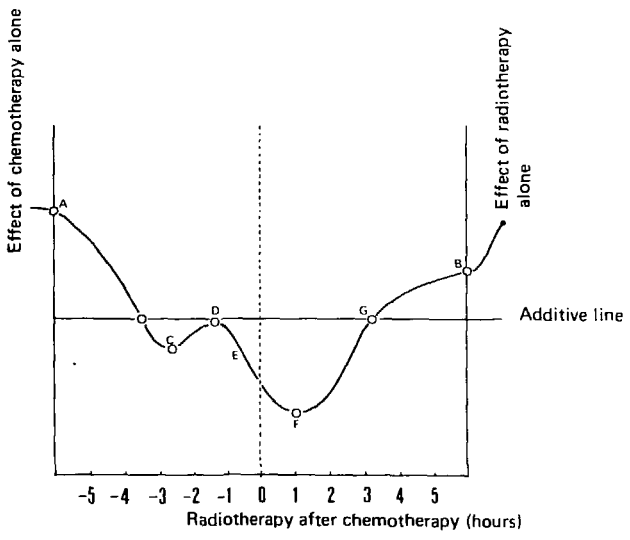
5. Experimental Investigations of Combination Treatment with Bleomycin and Radiotherapy.

The following investigators found in in vivo and in vitro studies, a supra-additive effect with combination treatment of Bleomycin and radiotherapy: Matsuzawa et al., 1972; Wharam et al., 1973; Terasima et al., 1975; Bistrovic et al., 1976 and Schrieve et al., 1979.

Other investigators (Bienkowska et al., 1973; Bleehen et al., 1974; Sakamoto and Sakka, 1974; Töllner and Haggeman, 1977) could demonstrate only an additive effect in their studies.

Matsuzawa et al. (1972) showed a supra-additive effect in an investigation of two cell lines. He found the characteristic biphasic survival curve with upward concavity with increasing concentrations of Bleomycin (Fig. 13). The dose-survival curve for radiotherapeutic treatment of the two cell lines produced the typical curve with a shoulder (Dq) which demonstrates sub-lethal recovery (Fig. 14). In this study,

Fig. 12



The survival fraction or clone-forming units are plotted along the ordinate, and the sequence of the administration of radiotherapy and chemotherapy are plotted on the abscissa. Below the line of additivity is the supra-additive area. The curve is explained as follows:

A. Cytotoxicity is determined only by the chemotherapeutic agent if the interval between radiotherapy and chemotherapy allows complete recovery after radiotherapy.

B. Cytotoxicity is determined only by radiotherapy if, due to the time interval between radiotherapy and chemotherapy, complete recovery from the chemotherapy has occurred.

C. Below the line of additivity is the area of supra-additivity. Supra-additivity may arise from radiation-induced arrest in chemotherapy sensitive phase of the cell cycle.

D. The effect is purely additive. No synchronization has occurred at the time of administration of chemotherapy.

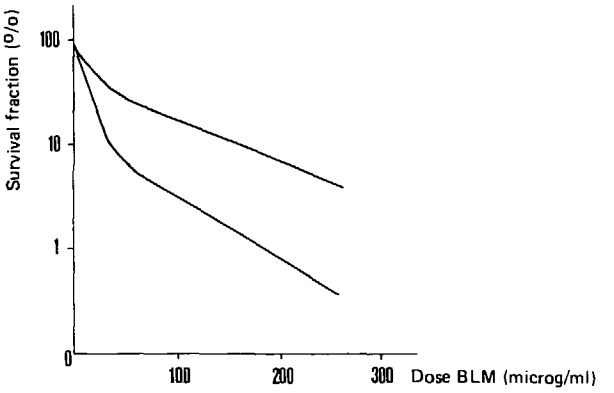
E. Negative slope in the curve due to either incomplete or inhibition of repair of sub-lethal radiation damage (SLD) by chemotherapy, and, thereby, of fixation of SLD.

F. Chemotherapy causes in this situation, radio-sensitization by the cytostatic drug, inhibition of DNA synthesis, and thereby, of DNA repair.

G. After complete recovery from chemotherapy, the curve rises to the level of radiotherapy alone.

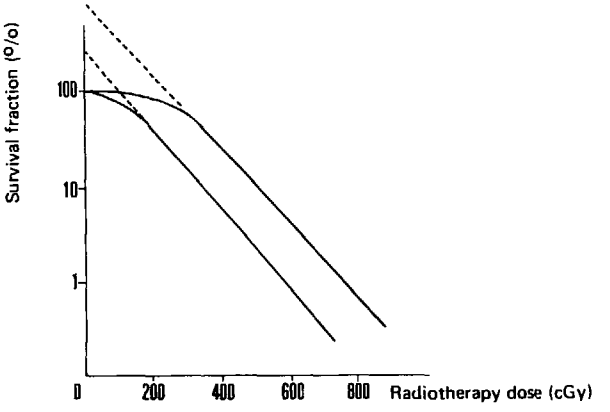
In practice, combination of the various phenomena result in a smooth curve.

Fig. 13



Dose-survival curve of two cell lines after treatment with Bleomycin.
FM3A (upper curve)
C2W (lower curve)
(Matsuzawa, 1972)

Fig. 14

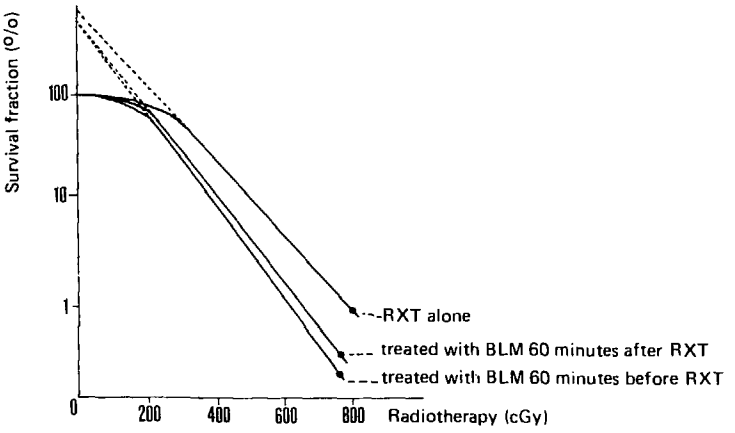


Dose-survival curve of two cell lines after treatment with radiotherapy.
C2W (upper curve)
FM3A (lower curve)
(Matsuzawa, 1972)

the D_0 * of the radiation dose-survival curve decreased distinctly after incubation with Bleomycin (Fig. 15), which demonstrated the supra-additive effect. Using incubation of

* The D_0 is a measure of the slope of a dose-survival curve in the exponential part of the curve. The D_0 is the quantity of chemotherapeutic agent or radiotherapy which is necesssary to reduce the survival fraction by e^{-1}

Fig. 15

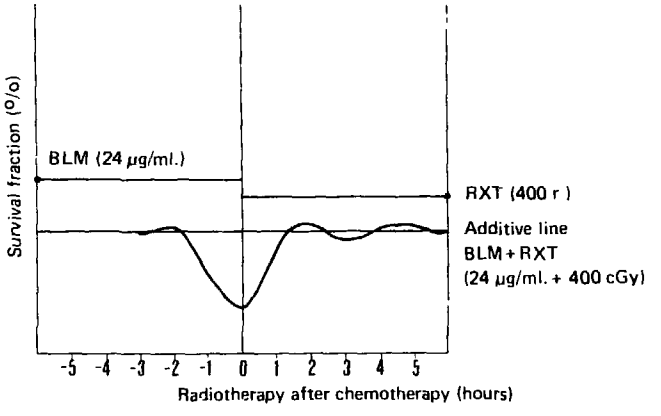


RXT – Radiotherapy
BLM – Bleomycin (25 µg/ml for 1 hour)

Survival curve of C2W cell line (Matsuzawa, 1972)
: D_0 decreases with combined treatment

the C2W cell line with Bleomycin followed by irradiation (400 cGy). Matsuzawa showed that the greatest supra-additive effect was obtained with simultaneous treatment using both treatment modalities (Fig. 16). The supra-additive effect was observed if treatment intervals did not exceed 2 hours (Fig. 16).

Fig. 16



Effect of the sequence of a combination treatment of radiotherapy and chemotherapy (Bleomycin) on a C2W cell line (Matsuzawa, 1972).

Schrieve et al. (1979) carried out their research with EMT6 tumour cells in vitro and found a change in (Dq) of the survival curve if the cells were treated simultaneously with Bleomycin. The D_0 of the combined treatment curve decreased by a factor of 1.3 with respect to the dose- survival curve for radiotherapy alone. As far as the sequence is concerned, it was evident that simultaneous administration caused the greatest supra-additive effect, which was observed by Schrieve et al. (1979) between the +1 and -4 hours in the sequence curve.

By contrast, Sakamoto and Sakka (1974) were unable to observe changes in the D_0 The survival curve with pre-treatment with Bleomycin was below the curve for radiotherapy alone. On the basis of these findings they concluded that there was only a "possibility" of an additive effect. The difference in sequence between Bleomycin administered 1 hour before radiotherapy or directly after radiotherapy caused no differences in survival in Sakamoto's research.

Concerning the sequence, Jørgensen (1972 a,b.) reached the conclusion that simultaneous administration was associated with a greater reduction in tumour volume than after a sequential regime if Bleomycin and radiotherapy were administered with an interval of more than 48 hours.

Twentyman et al.(1979) investigated inhibition of tumour infiltration using 3 murine tumours. He applied various sequences of the combination treatment (Bleomycin and radiotherapy) and found no difference in tumour growth inhibition in relation to the various sequences. The inhibitory effect was additive. From these in vivo studies, it was concluded that in vivo several other factors interfere with the effectiveness of the treatment, rendering the determination of the sequence optimum difficult, if not impossible.

To summarize, various studies have shown that combination treatment with Bleomycin and radiotherapy:

a. yielded at least an additive effect, and in many cases a supra-additive effect with sensitive cell lines.
b. resulted in optimal sequence of the combination treatment only in vitro.
c. showed a supra-additive effect only if time interval did not exceed a few hours.

6. Experimental Investigations of Combination Treatment with Adriamycin and Radiotherapy.

Fu et al. (1979), Harris and Schrieve (1979) and Siemann and Sutherland (1980) were unable to demonstrate a supra-additive effect of combination treatment in their experimental models.

Fu et al. (1979) used 3 different models of the EMT6 murine tumour in vivo:
1. solid (1 cm diameter in the flank)
2. visible metastases (lung)
3. microscopic metastases (24 hours after inoculation).
After combination treatment of Adriamycin and radiotherapy, there was no change in the D_0 in all three situations.

Harris and Schrieve (1979) examined EMT6 tumour cells in vitro. After combination treatment of Adriamycin and radiotherapy, it was concluded that the D_0 did not decrease.

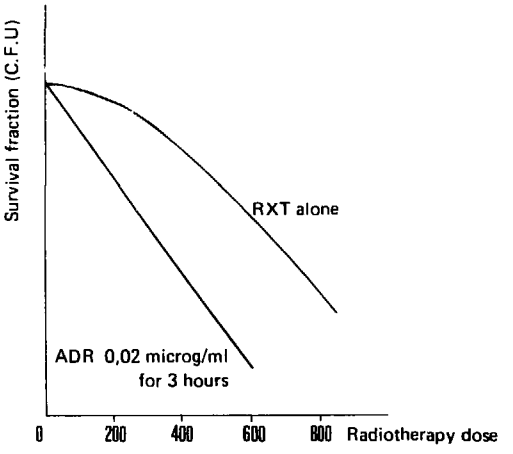
Siemann and Sutherland (1980), used EMT6 tumours in vivo, to measure the effect of combination treatment of Adriamycin and radiotherapy. It was concluded that the best result from the combination treatment was within the addition envelope and can therefore be judged, at best, as additive.

In contrast to the findings of the three mentioned authors, other investigators did demonstrate supra-additive effects.

Poulakos et al. (1975) performed an in vitro study with a P815X2 murine mastocytoma, and measured tumour volumes during and after treatment: The combination of Adriamycin and radiotherapy treatment was the most effective regime compared to treatment with Adriamycin or radiotherapy alone.

Bistrovic et al. (1978) investigated a 929 E murine cell line. Survival after irradiation (D_0) decreased after incubation with Adriamycin (Fig. 17). If Adriamycin was

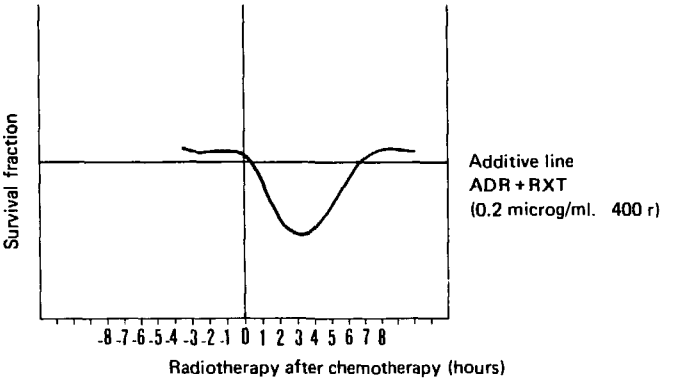
Fig. 17



Dose-survival curve of 929E murine cell line (Bistrovic, 1978), after treatment with radiotherapy (upper curve), and after incubation with Adriamycin plus radiotherapy (lower curve).

administerd a few hours after irradiation, the supra-additive effect was maximal (Fig. 18).

Fig. 18



Supra-additive effect on cell death using combination treatment of radiotherapy plus chemotherapy with Adriamycin (Bistrovic, 1978).

Other investigators reached the same conclusion concerning the radiation dose-survival curve after incubation with Adriamycin (Hellman and Hannon (1976), Phillips and Fu (1976) and Belli and Piro (1977).

Byfield et al. (1977) were able to observe D_0 changes in the radiation dose-survival curve of HeLa cell lines after incubation with Adriamycin. They showed that the extent of the additive effect depended upon the individual sensitivity of the cell line to Adriamycin. The increased toxicity of the combination treatment was explained as follows: single DNA helix breaks caused by Adriamycin and will lead to increased double (lethal) DNA strand breaks.

Braunschweiger et al. (1981) reached the conclusion, after an in vivo study with an anaplastic T1699MT carcinoma utilising combination treatment with Adriamycin and radiotherapy, that:
1. The inhibition of tumour growth is dose-dependent for both Adriamycin and radiotherapy.
2. The effect of combination treatment is additive as far as the growth inhibition of the tumour is concerned.
3. A supra-additive effect in the growth inhibition is detectable when the sequence between the radiotherapy and chemotherapy administration is optimal, i.e. if the second treatment modality is administered before complete recovery from the cell cycle changes after the first treatment has occurred.

To summarize, most investigators registered in sensitive cell lines, a supra-additive effect if Adriamycin and radio-

therapy were administered at optimal time intervals and optimal sequences.

7. Experimental Flow Cytometric Investigations after Combined Administration of Chemotherapy and Radiotherapy.

Linden et al. (1973) and Reddy et al. (1977) used L929 murine fibroblasts in their experiments. Linden et al. (1973) irradiated a culture with 9 x 25 cGy at intervals of 3 hours and saw a massive accumulation of cells in the G_2+M-phase (from 17.4 to 71.8%). A cell line incubated with Adriamycin caused a similar accumulation of cells in the G_2+M-phase (from 17.4 to 74.5%). The combination of both treatment modes resulted however, in a lower accumulation of cells in the G_2 +M-phase (even lower than that which either treatment would attain independently). In an investigation by Reddy et al. (1977) an additive accumulative effect was shown to occur.

In studies with cell lines it was not conclusively shown that synchronization effects of the separate treatment modalities could be added when the modalities were combined. This is logical because inhibition of cell cycle progression can interfere with the cytotoxic effects of one of the treatment modalities to such an extent that the other modality cannot effect an accummulation.

CHAPTER VII

Literature Review of Intra-arterial Infusion of Tumours in the Head and Neck Area

1. Introduction.
2. Intra-arterial Palliative Monochemotherapy.
3. Intra-arterial Palliative Polychemotherapy.
4. Intra-arterial Chemotherapy as Induction Chemotherapy, and Intravenous Induction Chemotherapy.

4.1. Introduction.
4.2. Biological Selection.
4.3. Randomized Studies of Intra-arterial Induction Monochemotherapy.
4.4. Intra-arterial Induction Polychemotherapy.
4.5. Intravenous Induction Chemotherapy; Randomized Trials with Methotrexate Monochemotherapy.
4.6. Intravenous Induction Polychemotherapy.

LIST OF ABBREVIATIONS USED IN THE TABLES OF LITERATURE STUDY

ADR	Adriamycin (Doxorubicine)
BLM	Bleomycin
Cis.pl.	Cisplatinum (C.D.D.P)
Cyclo. ph.	Cyclo phosphamide
5 Fu	5 Fluorouracil
MTX	Methotrexate
Vi.	Vincristine
C.B.M. regime	Cis.pl. + BLM + MTX
V.B.M. regime	Vi. + BLM + MTX
C.O.B. regime	Cis.p. + Vi. + MTX
leucov. resc.	leucovorin rescue
i.a.	intra-arterial
i.v.	intravenous
i.m.	intramuscular
cont. inf.	continuous infusion
intermitt.	intermittent
ex. car. a.	external carotid artery
hr(s)	hour(s)
wk(s)	week(s)
mth(s)	month(s)
yr(s)	year(s)
gr	gram
mg	milligram
pts	patients
CHT	chemotherapy
RXT	radiation therapy
fx	fractions
H and N CA.	squamous cell carcinomas of the head and neck
s	surgery
r = cGy	RAD = centi Gray
CR	complete response (rate)
CR's	complete responders
PR	partial response (> 50%) (rate)
PR's	partial responders (> 50%)
NR	no response (rate)
NR's	non responders
N.E.D.	no evidence of disease
A - N.E.D.	alive - no evidence of disease
D - N.E.D.	dead - no evidence of disease
F.O.D.	free of disease
A - F.O.D.	alive - free of disease
D.W.D.	death with disease
REC.	recurrence
MST	mean survival time
DFS	disease-free survival

1. Introduction.

Intra-arterial infusion chemotherapy was first carried out by Klopp et al. (1950) and Bierman et al. (1951, 1956), who used prolonged intermittent infusion. Continuous intra-arterial infusion was carried out by Sullivan et al. in 1953. Creech et al. (1958) introduced extracorporal (isolated) perfusion to chemotherapy. With this technique, very high concentrations can be attained in the perfused area, the loss of the cytostatic agent to the rest of the body via the collateral circulation being prevented by the use of a tourniquet (Didolkar et al, 1978). In the head-neck area extracorporal perfusion can not be used because of the large number of collateral vessels.

Sullivan et al. (1959) treated head-neck tumours with continuous intra-arterial infusion of MTX and intramuscular leucovorin rescue. It was established that daily intra-arterial bolus injections did not result in a therapeutic advantage when compared to intravenous pulse administration, and subsequently intra-arterial continuous infusion for 6-10 days was used.

Later, it appeared that intermittent prolongued intra-arterial infusion, (Goldsmith and Carter, 1975) produced better results that continuous intra-arterial infusion. This is sufficient reason to assume that not only the administration route but also the administration regime is important in determining the final effect on the tumour.

Many authors have found a therapeutic advantage of intra-arterial administration compared with intravenous administration of cytostatic agents, for example Espiner (1966), Donegan and Harris (1972), Oberfield et al. (1973), Goldsmith and Carter (1975), and Bertino et al. (1975). This effect was particularly evident when the responders were also treated with radiotherapy and/or surgery (Eschwege and Gary-Bobo 1978; Cruz et al., 1974; Curioni and Quadu, 1978; Stephens Mosely, 1981; Caracciolo et al., 1983).

Intra-arterial infusion was first used as palliative therapy, with varying results. The number of patients in which complete response occurs after prolonged (intermittent) intra-arterial infusion with MTX varies between 0% (Acquarelli et al., 1964) and 28% (Sullivan and Watkins, 1965), with an average of 17% (Table I). Investigators in Table I recorded complete or partial (greater than 50%) response in 70% of cases. The duration of the response was, however, disappointingly short.

None of the investigators who later administered MTX intravenously (Table II) recorded a case of complete response, and partial (PR greater or equal to 50%) response was obtained in 43% of the patients treated. This indicates that

TABLE I RESULTS OF CONTINUOUS INTRA-ARTERIAL MTX INFUSION (AS PALLIATIVE THERAPY)

AUTHOR	ADMINISTRATION ROUTE	DOSE SCHEDULE	PATIENTS EVALUATED	RESPONSE RATE CR	RESPONSE RATE PR	REMARKS SURVIVAL
Sullivan (1961)	i.a. infusion direct into the ex.car.art.	MTX 50 mg/day with leucov.resc. 3 mg 4x/day i.m. for 7-30 days	27	6/27 22%	21/27 77%	
Sullivan (1962)	"	"	42	8/42 19%		
Sullivan (1965)	"	"	72	20/72 28%	35/72 48%	
Westburry (1962)	i.a. cont.inf.	MTX 50 mg/day 5-6 days without leucov.resc.	26	3/26 12%		all patients had preoperative RTX poor prognosis group
Espiner (1962)	i.a. cont.inf.	MTX 50 mg/day with leucov.resc. 18 mg/day for 5-6 days	23	4/23 18%	.2/23 52%	duration of response < 3 mths.
Espiner (1964)	"	"	75	11/75 14%	45/75 60%	duration of response was short
Acquarelli (1964)	i.a. cont.inf.	MTX 50 mg/day with leucov.resc. 4 mg 6x/day i.m.	30	0/30 0%	8/30 27%	
Watkins (1964)	i.a. cont.inf.	MTX 50 mg/day with leucov.resc. 6 mg 4x/day i.m. duration of infusion until max. toxicity	68	15/68 22%	27/68 40%	CR's had longer remission
Burn (1969)	i.a. cont.inf.	MTX 50 mg/day with leucov.resc. 6 mg/day i.m.	45	4/45 8%	21/45 46%	
Couture (1968)	i.a. cont.inf.	MTX 50 mg/day with leucov.resc.	28	1/28 3%	16/28 57%	all patients had oral cancer

TABLE II RESULTS OF CONTINUOUS INTRAVENOUS MTX INFUSION (AS PALLIATIVE THERAPY)

AUTHOR	ADMINISTRATION ROUTE	DOSE SCHEDULE	PATIENTS EVALUATED	RESPONSE RATE CR	RESPONSE RATE PR	REMARKS SURVIVAL
Lefkowitz (1967)	i.v. cont.inf.	MTX 1-3 mg/kg/day with leucov.resc. 6 mg 2x/day i.m.	18	0	4/18 22%	
McComb (1967)	i.v. intermitt. cont.inf.	MTX 2 mg/kg/day every 5th day with leucov.resc. 6 mg 4x/day	19	0	6/19 32%	
Capizzi (1970)	i.v. intermitt. cont.inf.	MTX 240 mg/m^2/day every 4th day with leucov.resc. 75 mg i.v. in 12 hrs followed by 12 mg i.m. 4x/day	21	0	13/21 61%	
Levitt (1972)	i.v. cont.inf. (36-42 hrs) every 2nd wk	MTX 360-1080 mg/m^2 with leucov.resc. 40 mg/m^2 i.v. in 6 hrs followed by 25 mg P.O. 4x/day	25	0	13/25 52%	

TABLE III RESULTS OF CONTINUOUS INTERMITTENT INTRA-ARTERIAL ADR INFUSION

AUTHOR	ADMINISTRATION ROUTE	STAGING SITE NUMBER	DOSE SCHEDULE
Di Pietro (1973)	i.a. intermitt. cont.inf. (6-12 hrs.)	advanced disease 8	ADR 0·3 mg/kg/day 15 days TD 120-150 mg

RESULTS OF CONTINUOUS INTERMITTENT INTRA-ARTERIAL BLM INFUSION

Höltje (1976)	i.a. intermitt. cont.inf. (3 hrs)	advanced disease oral cavity 35	BLM 15 mg/day 5x/wk 1st wk 4x/wk 2nd wk 3x/wk 3rd wk sometimes MTX 25 mg i.v. during interruptions, sometimes followed by S and/or RXT
Bilder (1974)	i.a. regional cont.inf.	H and N advanced disease 10	5 pts BLM 10 mg/day TD 100-150 mg randomised with 5 pts MTX 10 mg/day followed by BLM 10 mg/day followed by MTX etc.
Molinari (1985)	i.a. intermitt. cont.inf. (6-12 hrs)	all sites T_2 + bone involvement T_3 without bone involvement 33	BLM 15 mg/day 13 days TD 150-195 mg followed by RXT and/or S

RESPONSE RATE		REMARKS SURVIVAL
CR	PR	
0	3/8 38%	ADR gave a worse response than other i.a. monodrug therapies H and N CA responded best
17/35 49%	10/35 29%	after a follow up of 9-34 mths 8/17 CR 47% A.NED 7/17 CR 41% mortality from intercurrent disease
0	4/5 80%	multidrug CHT gave more CR and a higher CR + PR rate
3/5 60%	2/5 40%	
37%		

TABLE IV RESULTS OF CONTINUOUS INTRA-ARTERIAL INFUSION WITH A MULTIDRUG CHEMOTHERAPY REGIME (AS PALLIATIVE THERAPY)

AUTHOR	ADMINISTRATION ROUTE	*STAGING* SITE NUMBER	*DOSE SCHEDULE*	*RESPONSE RATE* CR	PR	REMARKS SURVIVAL
Oberfield (1973)	i.a. local-selective cont.inf.	advanced oral cavity 46	deoxy-5 fluorouridine 1 mg/day MTX 50 mg/day with leucov.resc. 6 mg 4x/day i.m. 5 Fu 5 mg/kg/day for weeks to months with interruptions	12/46 25%	21/46 45%	survival > 1 yr CR: 13/24 54% PR: 9/49 31% duration of remission > 1 yr
Donegan (1972)	i.a. cont.inf.	all sites all stages 113	88 pts 5 Fu i.a.pulse 4 pts BLM i.a. 26 pts MTX with leucov.resc. 8 pts MTX + 5 Fu	CR + PR 36% CR + PR 50% CR + PR 24% CR + PR 25%		oral cavity CA gave the greatest response rates 7/16 44% duration of the remission short: 60% < 2 mths
Freckman (1972)	i.a. cont.inf. 5-10 days ex.car.a. via car.a.	far advanced and REC all sites all kinds of tumours 169	36 pts 5 Fu 4 pts MTX 28 pts 5 Fu + MTX + Vi 2 pts 5 Fu + MTX + Vi + dactinomycine	CR + PR 12/26 33% CR + PR 1/4 25% CR + PR 15/28 53% CR + PR 2/2 100%		survival after follow up CR: 16.9 mths NR: 4.4 mths CR rates > previously untreated multidrug CHT gave a better CR + PR rates compared with monodrug CHT

intra-arterial administration offers a therapeutic advantage in the direct response to MTX.

2. Intra-arterial Palliative Monochemotherapy.

Di Pietro et al. (1973) administered a continuous intra-arterial infusion of ADR and found no cases of complete response, and partial response in only 3 of the 8 patients (38%). It is thus evident that Adriamycin is not suitable for monochemotherapy in carcinoma of the head/neck area.

Höltje et al. (1976) evaluated a series of 35 patients after intra-arterial infusion with BLM and found complete response in 17 of the 35 patients (49%).
Molinari (1985) found a complete response of 37% (Table III).

In summary, BLM is therefore superior to MTX and ADR as monochemotherapy in achieving complete response.

3. Intra-arterial Palliative Polychemotherapy.

Due to the moderate results of intra-arterial mono-chemotherapy, other investigators searched for better response rates with various polychemotherapy combinations.

Bilder and Hornova (1974) randomly selected a small number of patients (5 in each treatment category) and saw greater tumour reduction in the combination category (i.a. MTX + BLM sequentially) than in intra-arterial BLM monochemotherapy.
Oberfield et al. (1973) infused a combination of 5Fu and MTX intra-arterially, and found a complete plus partial response rate (70%) (Table IV), which did not differ from MTX monochemotherapy (70%) (Table I). Donegan and Harris (1972) were similarly unable to register better results with the same combination, than with monotherapy with either MTX or BLM. BLM monotherapy even proved to be superior to the combination of 5Fu and MTX.

Freckman (1972) saw however a clear increase in the response rate using intra-arterial polychemotherapy with 5Fu, MTX, Vi and Dactinomycin in comparison to intra-arterial monotherapy with 5Fu or MTX alone (but here also the numbers of patients in the study are limited).

A tentative conclusion from the findings of Bilder (1974), Oberfield et al. (1973), Freckman (1972) and Cruz et al. (1974), is that polychemotherapy offers a probable chemotherapeutic advantage. It is appropriate to refer to the animal study of Schouwenburg et al. (1980) (Ch. IX, 3) in which the clear superiority of combination chemotherapy was made evident.

4. Intra-arterial Chemotherapy as Induction Chemotherapy, and Intravenous Induction Chemotherapy.

4.1. Introduction.

The improved effectiveness of chemotherapy in squamous cell carcinoma in the head-neck region has allowed this disease to be approached by initial chemotherapy to reduce the size of the primary tumour (stage reduction) such that the primary tumour becomes resectable with curative intent.

In most randomized controlled clinical trials using induction chemotherapy in one treatment category and standard procedures in the other treatment category, Stage III and IV (AJC 1978) head-neck tumours are selected for treatment. However, this staging system contains so many sub-groups (Chapter I, 4.2 - 5.7), that the clinical therapeutic effect of the respective treatment categories can be evaluated only with difficulty. It is likely that demonstration of possible therapeutic advantage in adjuvant chemotherapy cannot be demonstrated because of the great heterogeneity of the patient material, particularly in evaluation of the longer survival period. The results of most randomized studies using induction chemotherapy followed by radiotherapy as one treatment category, as opposed to radiotherapy alone in the other, must also be evaluated in the light of the above considerations.

4.2. Biological Selection.

When the patient material is selected according to tumour response to induction chemotherapy, this biological selection is certainly an acceptable means of prognosis. Authors who were able to observe biological selection and recommend induction therapy as a good criterion for further therapy, include Gollin and Johnson (1971), Freckman (1972), Oberfield et al. (1973), Snow and Sindram (1973)), Cruz et al. (1974), Richard and Snow (1975), Höltje (1976), Eschwege and Gary-Bobo (1978), Curioni and Quadu (1978). They all found a correlation between the extent of response and the average increase in short term (less than 2 years) survival. If induction chemotherapy produces no response, Gillis et al. (1982) refers to a "prognostically poor indication".

Examples of biological selection have been presented by, among others, Snow and Sindram (1973). They evaluated 56 patients and found a correlation between response to chemotherapy and cure rate. Of the patients who responded to induction chemotherapy, one third were living and tumour-free after the follow-up period; of the non-responders, less than one sixth were living and tumour-free.

Eschwege and Gary-Bobo (1978) found a significantly better

short-term (greater than 3 years) survival rate of 45% in patients with a good response after intra-arterial induction chemotherapy plus follow-up treatment with radiotherapy and/or surgery, than for the whole patient group treated (22%). Curioni and Quadu (1978) evaluated 27 Stage IV head-neck carcinomas after intra-arterial polychemotherapy as induction treatment (Table VI). The mean survival time of the responders to polychemotherapy treatment plus follow-up treatment with radiotherapy and/or surgery, was 51.4 months, while the mean survival period of the whole patient group was 5.4 months.

4.3. Randomized Studies of Intra-arterial Induction Monochemotherapy.

In the research of Richard et al (1974 and 1975, Table V) there was a significant improvement in the short (less than 18 months) survival in patients with T_4 tumours treated with MTX followed by radiotherapy, as compared to the treatment category of radiotherapy alone.

Nervi et al (1978) was able to demonstrate a significantly improved survival rate in the combination therapy category (MTX followed by radiotherapy) only for oral cavity carcinomas (Table V). Gollin and Johnson (1971) applied intra-arterial induction chemotherapy with 5Fu followed by radiotherapy in one treatment category of his study, and radiotherapy alone in the other category (Table V). Although there was an improvement in the complete response rate for the combination treatment category (76%) in contrast to the radiotherapy category (55%), no improvement in 5 year survival rate was demonstrated. However, there was again a significant difference, when the group of good responders was considered separately (biological selection).

4.4. Intra-arterial Induction Polychemotherapy.

Different investigators have obtained varying response rates after induction chemotherapy:

Cruz (1974)	CR 15%	(CR + PR) = 90%	(5Fu; MTX; Vi)
Curioni (1978)	CR 12.5%	(CR + PR) = 47%	(Vi; MTX; BLM; ADR; Mytomycin)
Szabo (1979)	CR 6%	(CR + PR) = 100%	(Vi; BLM; MTX)

Although the response ratio is higher than for monochemotherpy, the complete response rate is disappointingly low. The local control percentages are clearly better after follow-up treatment with surgery and/or radiotherapy. Eschwege and Gary-Bobo (1978), Cruz et al. (1974), Curioni and Quadu (1978), Stephens Moseley et al. (1981) and Caracciolo et al. (1983) have demonstrated this in

TABLE V RESULTS OF INTRA-ARTERIAL MTX INFUSION AS INDUCTION CHEMOTHERAPY

AUTHOR	ADMINISTRATION ROUTE	STAGING SITE NUMBER	DOSE SCHEDULE
Bilder (1968)	i.a. local selective cont.inf.	all sites all stages 17	9 pts MTX 30-15 mg/day 7 days without leucov.resc. 8 pts MTX 15-30 mg/day 7 days without leucov.resc. followed by RXT TD 3-6000 r
Bilder (1970)	"	all sites all stages 42	MTX 7.5 mg/day 7 days followed by RXT TD 3-6000 r (18 pts) or S (23 pts)
Desprez (1970)	i.a. cont.inf.	all sites all stages 103	MTX 50 mg/day + leucov.resc. 6 mg 4x/day 10 days followed by S (after 3-6 wks)
Snow (1973)	i.a. cont.inf.	oral cavity CA 80	MTX 50 mg/day for 7-10 days followed by RXT TD 6-6500 r S
Sealy (1974)	i.a. cont.inf.	all sites all stages 10	MTX 50 mg/day 7 days + leucov.resc. 4-6 mg i.m. 4 x/day followed by RXT 500 r 9 fx/4½ wks TD 4500 r
Richard (1974)	i.a. intermitt. cont.inf. (10-12 hrs)	oral cavity oral pharynx T4 39	21 pts MTX 50 mg/day + leucov.resc. 15 mg/day for 8-10 days 14 days interruption followed by RXT TD 3-6000 r (fx 300r) randomized with 18 pts RXT alone
Nervi (1978)	i.a. cont.inf.	stages II + III + IV all sites 82	42 pts MTX 3-5 mg/day for 7-12 days followed by RXT 200 r 5 fx/wk TD 6500 r randomized with 40 pts RXT alone

RESPONSE RATE		REMARKS SURVIVAL
CR	PR	
1/9 11%	6/9 66%	low doses gave more CR
2/8 25%	1/8 13%	
8/42 19%	6/42 14%	
		lower doses gave the same effects on the tumor
28/103 27% 19/28 T_1 T_2 9/28 T_3 T_4 4/28 N+	75/103 73% 4/ 75 T_1 T_2 34/ 75 T_3 T_4 35/ 75 N+	survival rate of up front CHT gave no better response rates than hist.contr.group without CHT
	60-75%	56 were evaluated PR $\geq$ 50% A.NED 33% PR $<$ 50% A.NED $<$ 16% 15 patients failed to complete therapy due to catheter complications 5% due to systemic toxicity
2/10 20%		follow up $>$ 1 yr LFD 2/10 oral carcinomas had better results than other locations
		short term survival rate ($<$ 18 mths) was better than for induction CHT with RXT alone (no significant difference between the two groups after 3 yrs)
		remission rate was correlated with survival rate
		for oral cavity CA there was a significantly better survival rate and L.FOD rate in the induction CHT group
		the worse the staging the poorer the response

AUTHOR	ADMINISTRATION ROUTE	STAGING SITE NUMBER	DOSE SCHEDULE
Eschwege (1978)	i.a. cont.inf.	previously untreated 42 pts previously treated 45 pts	MTX 50 mg/day for 8-12 days or BLM 7½-15 mg/day TD 120-200 mg followed by RXT TD 3-5000 r S in case of bone invasion
Molinari (1985)	i.a. intermitt. cont.inf.	all sites T_2 + bone involvement T_3 without bone involvement 10	MTX 50 mg/day 10 days + leucov.resc. TD 500 mg followed by RXT and/or S

RESULTS OF INTRA-ARTERIAL 5 FU INFUSION AS INDUCTION CHEMOTHERAPY

Gollin (1971)	i.a. cont.inf.	all stages oral cavity CA 64	32 pts 5 Fu 3-5 mg/kg/day for 8-27 days followed by RXT TD 6000 r in 6 wks randomized with 32 pts RXT alone TD 6000 r in 6 wks

their patient material (Table VI).

4.5. Intravenous Induction Chemotherapy; Randomized Trials with Methotrexate Monochemotherapy.

With respect to intravenous induction chemotherapy, the present study does not include a complete literature review. Several studies are however mentioned for both comparison with results after intra-arterial treatment and to indicate the role of combination induction chemotherapy.

Table VII shows several randomized studies with MTX as the intravenous induction chemotherapeutic agent as one treatment

RESPONSE RATE			REMARKS SURVIVAL
CR		PR	
4/87	4%	previously untreated pts 22/45 52% previously treated pts 15/45 33%	multidrug modality treatment gave better survival rates in cases responding to CHT
		7/10 70%	

(control of the primary tumour) 13/17 76%	MST 25.4 mths 5 yr survival rate 5/23
10/18 55%	MST 27.9 mths 5 yr survival rate 5/15
	no difference in survival rates of the 2 groups
	in the induction CHT group significantly better survival rate in the responders than non-responders

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category, and radiotherapy or surgery alone, as the other. None of the studies using MTX intravenous induction chemotherapy resulted in an improved survival rate after the respective follow-up periods:

Van Essen et al. (1968): 33% in both treatment categories
Knowelton et al. (1975): 22% in the combination category
: 31% in the radiotherapy category
Tarpley et al. (1975) : no difference in survival

As has already been stated in 4.1. above, randomized studies involving survival of patients with too great a number of staging subgroups, renders evaluation of the possible therapeutic gain difficult, if not impossible. Such considerations probably affect the survival rates in the

TABLE VI RESULTS OF INTRA-ARTERIAL INFUSION WITH A MULTIDRUG REGIME AS INDUCTION CHEMOTHERAPY

AUTHOR	ADMINISTRATION ROUTE	STAGING SITE NUMBER	DOSE SCHEDULE
Sealy (1972)	i.a. cont.inf.	H and N CA previously untreated pts	Nitrog.Must 2 mg/day 1 day MTX 50 mg/day + leucov.resc. Thiothepa 15 mg 2x
Cruz (1974)	i.a. cont.inf.	all sites all stages 41	5 Fu 15 mg/kg/day MTX 5 mg/day Vi 0.2 mg/kg/day in 15-16 days followed by RXT and S (N + neck)
Auersperg (1975)	i.a. cont.inf.	T3 T4 H and N CA 46	22 pts MTX 20-50 mg/day TD 650 mg + leucov.resc. 10 pts BLM 15-30 mg/day TD 360 mg 8 pts MTX followed by BLM 6 pts BLM 15 mg/day 3-4 days Vi 1 mg/day 1-2 days followed by MTX 25 mg/day 3-4 days followed by RXT
Curioni (1978)	i.a. intermitt. cont.inf. + i.a. bolus	stages III + IV all sites 40	Vi 1 mg/m^2 i.a. bolus MTX 80-120 mg/m^2 i.a. 12 hrs inf. BLM 10-15 mg/m^2 i.a. bolus ADR 10-20 mg/m^2 i.a. bolus Mytomycine 4 mg/m^2 followed by S and/or RXT
Szabo (1979)	i.a. cont.inf.	advanced disease all sites 49	Vi 0.5 mg/day BLM 15 mg/day MTX 15 mg/day after 2-3 wks interruption S

RESPONSE RATE		REMARKS SURVIVAL
CR	PR	
		follow up > 12 mths tongue/floor of mouth NED 6/30 20% lip/cheek NED 3/19 15% sinus NED 2/25 8%
after CHT 6/41 15%	30/41 75%	survival rate correlated with CR
after CHT + RXT 21/41 66%		
7/22 32%	11/22 50%	multidrug induction CHT + RXT showed better short term results (5-14 mths) than RXT alone
5/10 50%	4/10 40%	
2/8 25%	6/8 75%	
after CHT + RXT 5/6 83%	1/6 17%	
after CHT + RXT stage III 3/13 24%	4/13 30%	stage IV responders had a MST of 51.4 mths stage IV non-responders had a MST of 5.4 mths stage III tumours had a higher CR rate
after CHT + RXT stage IV 2/27 7%	10/27 37%	
CR + PR stages III + IV →	19/40 47%	
after CHT 3/49 6%	46/49 94%	relatively few CR's after induction CHT alone regional metastases had worse response than tongue and larynx tumours

AUTHOR	ADMINISTRATION ROUTE	STAGING SITE NUMBER	DOSE SCHEDULE
Stephens Moseley (1981)	i.a. cont.inf. 9 days	stages III + IV 13	BLM 0.75 U/kg every 2nd day MTX 0.5 mg/kg/day followed by RXT 200 r 5 fx/wk TD 5-6000 r followed by S 4-6 wks after RXT
Caracciolo (1983)	i.a. cont.inf.	oral cavity CA all stages 37	11 pts ADR + MTX 26 pts MTX followed by RXT
Molinari (1985)	i.a. intermitt. cont.inf.	all sites T2 + bone involvement T3 without bone involvement Vi + BLM 65 Vi + BLM + MTX 42	Vi + BLM 1 1 day 12 days 1.5.9 Vi + BLM + MTX 1 1 1 day day day 1.6.11 1.4 5.10.15 6.9 11.14 Vi 1 mg/day BLM 15 mg/day MTX 50 mg/day

TABLE VII RESULTS OF INTRAVENOUS MTX INFUSION AS INDUCTION CHEMOTHERAPY

AUTHOR	ADMINISTRATION ROUTE	STAGING SITE NUMBER	DOSE SCHEDULE
van Essen (1968)	i.v. pulse	stages II + IV cheek CA 75	25 pts MTX 0.2 mg/kg/day followed by RXT 25 pts 5 Fu 15 mg/kg/day followed by RXT 25 pts RXT alone TD 6500 r in 6 wks
Knowelton (1975)	i.v. cont.inf.	all sites all stages 40	20 pts MTX 0.2 mg/kg/day 5 days TD 240 mg/m^2 followed by RXT TD 4500-5000 r randomized with 20 pts RXT alone

RESPONSE RATE		REMARKS SURVIVAL
CR	PR	
after CHT+ RXT 4/13 30% contr. disease after CHT+ RXT+ S 10/13 77% contr. disease		no evidence of tumour in 77% of resection specimens 3/13 histologically showed incomplete resection
after CHT 2/37 5.4% after CHT + RXT 17/37 46%	23/37 62%	after 21.8 mths follow up 10/37 27% survival 27/37 73% mortality
	27/65 42%	
	31/42 74%	

RESPONSE RATE		REMARKS SURVIVAL
CR	PR	
14/17 82% after CHT + RXT		3/16 19% survival rate
12/18 67%		6/18 33%
8/15 53%		5/15 33%
		LFD 22.9%
		LFD 31.2%
		no difference in survival and CR rate

AUTHOR	ADMINISTRATION ROUTE	STAGING SITE NUMBER	DOSE SCHEDULE
Tarpeley (1975)	i.v. cont.inf.	all sites all stages operable 66	30 pts MTX 240 mg/m^2 + leucov.resc. 12 mg 4x/day followed by S randomized with 20 pts S slone
Taylor (1978)	i.m.	all sites stage III 7 pts stage IV 10 pts 17	MTX 60 mg/m^2 4x/day i.m. in 3 cycles day 1,5,9 + leucov.resc. followed by 2 wks. interruption followed by RXT and/or S
Ervin (1981)	i.v. push	all sites stages III + IV 21	MTX 3 mg/m^2 in 4 cycles + leucov.resc. followed by RXT and/or S

RESULTS I.M. BLM AS INDUCTION CHEMOTHERAPY

Rygård (1979)	i.m.	all sites all stages 68	I BLM 10-15 2-3 x/wk followed by II RXT 160-200 r 5 fx/wk followed by III S (if no CR occurs after I and II) 26 pts CR + PR 42 pts NR

RESPONSE RATE		REMARKS SURVIVAL
CR	PR	
after CHT	23/30 77%	REC rate 15/30 50% after an average of 10 mths
		REC rate 14/30 46% after an average of 7 mths
		no difference in survival rate
after CHT	8/10 80%	follow up > 20 mths REC 3/17 18% NED 11/17 65% no correlation between response and survival
after CHT	11/30 37%	after an average follow up of 38 mths the responders were A.FOD in 55%
		the whole group showed an average rate of A.FOD of 33%
		MST of responders 38 mths MST of non- responders 15 mths
		CHT showed a higher short time survival rate
after I + II 16/26 62% after I + II + III 20/26 77%		MST of CR + PR's 46 mths
after I + II 14/42 33% after I + II + III 22/42 52%		MST of NR's 15 mths

TABLE VIII RESULTS OF INTRAVENOUS MULTIDRUG CHEMOTHERAPY AS INDUCTION CHEMOTHERAPY

AUTHOR	ADMINISTRATION ROUTE	STAGING SITE NUMBER	DOSE SCHEDULE
Hong (1979)	i.v. push + i.v. cont.inf.	stages III + IV inoperable non-resectable 39	Cis.pl. 120 mg/m^2 i.v. cont.inf. day 1 followed by BLM 15 mg/m^2/day day 3-9 i.v. push Cis.pl. 120 mg/m^2 day 22 followed by S 19/39 pts and/or RXT
Shapshay (1980)	"	stages III + IV inoperable non-resectable oral CA 17	"
Glick (1980)	"	stages III + IV non-resectable all sites 29	"
Pennacchio (1982)	"	stages III + IV inoperable non-resectable all sites 23	23 pts " hist.contr.group
Gillis (1982)	"	advanced H and N all sites 77	24 pts (as above) + S + RXT 23 pts CHT (as above) + RXT 24 pts RXT alone

RESPONSE RATE		REMARKS SURVIVAL
CR	PR	
8/39 20%	22/39 56%	2/8 25% of resection specimens were tumour free 14/19 pts had non- resectable tumours
4/17 24%	8/17 47%	survival rate correlated with: a. CR b. T-staging c. tumour site d. N-staging
0%	12/25 48%	short term survival rate was better for responders than non-responders there were no differences between 5 yr survival rates for responders and non-responders
CR + PR 70%		CR's (after induction CHT) MST 62 mths NR's (after induction CHT) MST 5 mths
CR + PR 14%		CR's (after RXT alone) MST 16 mths NR's (after RXT alone) MST 3.3 mths
87%		DFS 35 mths MST 22 mths
60%		DFS 40 mths MST 14 mths
25%		DFS 3 mths MST 4.7 mths
after induction CHT alone 9/47 19%	25/47 53%	if CR occurs after induction CHT there is a significantly increased survival rate NR's after induction CHT is a bad prognostic indicator multi modality treatment showed 21% distant metastases during follow up

AUTHOR	ADMINISTRATION ROUTE	STAGING SITE NUMBER	DOSE SCHEDULE
Weaver (1980)	i.v.	stages III + IV all sites 57 oral cavity + tongue 40	COB-regime Cis.pl. BLM in 2 cycles followed by S and/or RXT
Sergeant (1981)	i.v.	stages III + IV inoperable all sites 45	Price-Hill-regime in 2 cycles with 2 wks interruption followed by RXT TD 7000 r
Petrovich (1981)	i.v. intermitt. cont.inf.	stage IV H and N CA	12 pts Vi 0.015 mg/kg/i.v. pulse MTX 50-100 mg/kg/i.v. (12 hrs infusion) in 2 cycles followed by RXT TD 7000 r primary 5000 r neck randomized with 11 pts RXT alone
Spaulding (1984)	i.v.	stages III + IV H and N resectable 50	COB-regime Cis.pl. 80 mg/m^2 i.v. day 1 Vi. 1.4 mg/m^2 i.v. day 2 BLM 15 U/m^2 day 3-7 in 2 cycles followed by 2 wks interruption followed by S (34/48)

studies of:
Van Essen et al. (1968): stages II to IV
Knowleton et al. (1975): all sites all stages
Tarpley et al. (1975) : all sites all stages

As shown for intra-arterial induction chemotherapy, many investigators could demonstrate a correlation between short-term survival and degree of response after intravenous administration (Table VII, Ervin et al (1981); Rygård and Hansen (1979); Table VIII, Shapshay et al. (1980); Glick et al. (1980); Pennacchio et al. (1982) (significant because of historical control); and Gillis et al. (1982); Weaver et al.

RESPONSE RATE		REMARKS SURVIVAL
CR	PR	
CR + PR → 46/57 80%		after induction CHT, significantly better survival rate for CR's than NR's
for oral cavity CA 10/40 25%	23/40 58%	
4/45 9%		follow up 1 yr A.FOD 33% follow up 2 yrs A.FOD 27%
after CHT + RXT 4/12 33%	4/12 33%	there were significantly better short term survival rates (< 2 yrs) in responders than non-responders
1/11 9%	3/11 27%	
11/50 22%	33/50 66%	REC occurring within 18 mths 12/47 26% 5/11 45% resection specimen were tumor free in downstaging after CHT from stage III + IV to stage II there wre no recurrences NED 65% after 42 mths follow up

(1980); Petrovich et al. (1981) and Spaulding et. (1984) (significant randomized study). Response is thus a prognostic parameter in induction chemotherapy because of the biological selection which occurs. This applies even more to complete responders. Ensley et al. (1984), Hollmann et al. (1984) and Szepesi et al. (1985) were able to demonstrate in their studies that those who responded fully to induction chemotherapy had a better survival than when the complete response was achieved by way of several treatment modalities.

4.6. Intravenous Induction Polychemotherapy.

Combined intravenous induction chemotherapy with Cisplatinum has until now produced the best response rate. The best results were obtained with Cisplatinum (100 mg/m^2) at the beginning of treatment and 5Fu (1000 mg/m^2 /day, as a continuous infusion over a 5 day period) in 3 cycles, each of 3 weeks' duration.

For comparative purposes, a review of the complete responses which were achieved with various Cisplatinum combinations, follows:

COB scheme (Cis pl. + Vi + BLM)
Weaver et al. (1980) CR 25%
Spaulding et al. (1982, 1984) CR 22%

Cisplatinum + BLM
Hong et al. (1979) CR 20%
Shapshay et al. (1980) CR 24%

Cisplatinum + 5Fu
Jacobs et al.(1984) 5Fu: 120 hr. cont. inf. 3 cycles CR 39%
Kies et al. (1984) CR 41%
Collins et al. (1984) CR 41%
Kish et al.(1984 a,b) 5Fu: cont 96 hr. inf. 2 cycles CR 20%
5Fu: 120 hr cont. inf. 3 cycles
CR 54% - (compl. resp.
Stage III 75%
Stage IV 49%)

In the light of the findings of the above mentioned investigators, there is strong justification for the use of induction chemotherapy in order to hold a choice of treatment modes in reserve for subsequent therapy. Such patients are treated with surgery and/or radiotherapy with curative intent. Whether surgery should be limited in terms of extent of resection and radiotherapy limited in terms of field size and dose because of prior tumour size reduction with chemotherapy, remains controversial. It is clear that induction chemotherapy is primarily of value as a biological selection criterion, because survival after surgery and radiotherapy is correlated with the degree of response to neo-adjuvant therapy. Thus patients who achieve complete response after chemotherapy, have relatively high survival rates as compared to those who achieve only a partial response. It remains to be determined whether this approach will in fact increase the total cure rate in groups of patients with advanced head and neck tumours.

The disadvantages of induction chemotherapy are the withdrawal of a patient from follow-up treatment when a complete response occurs (thus increasing the risk of local recurrence), and difficulty in determination of the resection

surfaces after induction chemotherapy, even with the use of precise staging and tattooing of the section edges under general anaesthesia and if determination of the tumour borders with advanced diagnostic techniques has been carried out.

CHAPTER VIII

LITERATURE REVIEW OF SIMULTANEOUS COMBINED CHEMOTHERAPY WITH RADIOTHERAPY AND SEQUENTIAL TREATMENT WITH CHEMOTHERAPY AND RADIOTHERAPY IN HEAD AND NECK TUMOURS

1. Introduction.

In the literature study on combined chemotherapy and radiation treatment, the clinical results of various investigators were compared by dividing the different treatment combinations into:
1. the phasing of the combination treatment (sequence of administration).
2. the different administration routes and administration method (a. bolus; b. continuous; c. intermittent continuous)
3. the cytostatic agent or combination of agents used.

The clinical studies can be divided into three groups:
a. Concomitant or concurrent administration. This term describes the concurrent or virtually concurrent administration of chemotherapy and radiotherapy. The treatment modalities may not, in principle, be separated by a period longer than the duration of the cell cycle (Tc).
b. Sequential administration. This term describes the alternating use of chemotherapy and radiotherapy in separate cycles. There is no question of a supra-additive effect in the narrow sense.
c. Induction or up-front chemotherapy. This term is used to describe pre-operative or pre-radiation administration of one or more cycles of chemotherapy to sterilize the peripheral tumour cells (this combination has already been discussed in Chapter VII).

Post-operative chemotherapy in single cycles or as maintenance dosing will not be discussed in this study, and neither will the palliative possibilities of chemotherapy. The problem in the literature study is to find conclusive evidence of a possible supra-additive effect after combination treatment, given the wide diversity in methods of treatment and evaluation. In the following tables, an attempt is made to bring some order to this complexity, so that tentative conclusions can be made.

2. Results of Concurrent Combined Administration of MTX and Radiotherapy.

Bagshaw and Doggett (1969) infused MTX intra-arterially as an intermittent continuous infusion; Mason and Ediger (1975), on the other hand, as an intra-arterial continuous infusion (Table I). The percentage of complete responses was 32% and 20% respectively. This could point to a possible advantage of intermittent continuous infusion. However, the number of patients in both studies was not large, and there are many variables in the tumour staging used in "advanced head and neck cancer." A definite conclusion can not be made on the grounds of this difference in response rate.

Randomized studies with the concurrent combination of MTX and

TABLE I RESULTS OF CONCURRENT COMBINED MTX ADMINISTRATION AND RADIOTHERAPY

AUTHOR	ADMINISTRATION ROUTE	STAGING SITE NUMBER	DOSE SCHEDULE
Bagshaw (1969)	i.a. intermitt. cont.inf. (8 hrs.)	advanced H and N CA 38	MTX 25 mg 18-43/days + leucov.resc. 24/mg/day RXT 200 r 5 fx/wk
Mason (1975)	i.a. cont.inf.	advanced H and N CA 30	MTX 50/mg/day i.a. cont. 6-10 days + leucov.resc. RXT 200 r 5 fx/wk TD 6000 r
Condit (1968)	i.v.	all sites all stages H and N CA 40	MTX 1-4 mg/kg every 2nd wk before RXT RXT 180 r 5 fx/wk or 500 r 3 fx/wk or 800 r 3 fx/wk
Lustig (1976)	i.v.	advanced disease 36	MTX 25 mg/day every 3rd day TD 125 mg RXT 200 r 5 fx/wk
Fazekas (1980)	i.v.	all sites stages III + IV 712	MTX 25 mg/day every 3rd day RXT 200 r 5 fx/wk TD 6500 r

radiotherapy as one treatment mode and radiotherapy alone as the other, show no clear therapeutic advantage of the combination mode. Bagshaw and Doggett (1969) found no therapeutic advantage (neither in complete response nor in survival) in the patients treated with the combination (Table I). Condit (1968) found only a slight advantage with the combination treatment. Lustig et al. (1976) were able to determine an increase of only a few months in the mean survival time (MST), with the use of concurrent combination treatment. Condit (1968) and Fazekas et al. (1980) showed an increased incidence of mucositis associated with the combination treatment, which resulted in 50% and 9% of the

RANDOMIZATION	RESPONSE RATE		REMARKS SURVIVAL
	CR	PR	
CHT + RXT 22 pts	7/22 32%		no difference in survival rate between the 2 groups
RXT 16 pts	6/16 38%		duration of remission in CR's is longer than NR's
	6/30 20%	10/30 33%	from the CR's 1 NED 4 yrs 1 NED 3 yrs 2 REC 1 yr 2 DWD 1-2 yrs
CHT + RXT 20	CR + PR → 9/10 90%		NED 5.1 mths
RXT 20	CR + PR → 12/15 80%		NED 3.4 mths
CHT + RXT i.v.	CR + PR → 5/36 14%		correlation between response rate and the 3 yr survival rate
CHT + RXT oral	CR + PR → 24/48 50%		survival rate same as with RXT alone there is a greater incidence of mucositis 9% had incomplete therapy because of this side effect

patients respectively not completing the therapy.

In summary, concurrent combined treatment with MTX and radiotherapy has not been proven to be more effective than radiotherapy alone.

3. Results of Concurrent Combined Administration of 5Fu and Radiotherapy

When the older intra-arterial studies of Jesse et al. (1964) and Latourette and Lawton (1963) (Table II) are compared with

TABLE II RESULTS OF CONCURRENT COMBINED INTRA-ARTERIAL 5 FU ADMINISTRATION AND RADIOTHERAPY

AUTHOR	ADMINISTRATION ROUTE	STAGING SITE NUMBER	DOSE SCHEDULE
Latourette (1963)	i.a. cont.inf.	advanced disease 9/24 all sites all stages 24	5 Fu 5 mg/kg/day RXT 200 r 5 fx/wk TD 6000 r
Jesse (1964)	i.a. cont.inf.	advanced disease 15	5 Fu 8 mg/kg/day for 10 days RXT 200 r 5 fx/wk
Sato (1970)	i.a. cont.inf.	T2 T3 max.sinus CA 57	5 Fu 250 mg/day for 21 days RXT 200 r 5 fx/wk on day 10 to 31 TD 7700 r S with PR
Goepfert (1973)	i.a. cont.inf	T3 T4 max.sinus CA 26	21 pts 5 Fu 6 mg/kg/day for 10 days RXT 200 r 5 fx/wk starting with i.a. inf. 3 pts MTX 50 mg/day followed by 5 Fu 10 days + RXT 2 pts MTX + leucov.resc. 15 days + RXT
Lawton (1972)	i.a. cont.inf.	advanced disease 50 CHT + RXT 35 CHT + RXT + S followed by S 15	5 Fu 5 mg/kg/day RXT 200 r 5 fx/wk

the intravenous study of Hall and Good (1961), no therapeutic advantage is shown as far as response rate is concerned.

There is a slight therapeutic advantage in intra-arterial administration, although in the intra-arterial studies only antrum tumours were treated and in the intravenous studies

RESPONSE RATE	REMARKS SURVIVAL
CR + PR 9/24 37%	3/9 had NED >1 yr oropharynx CA had most rapid remission but also the fastest recurrence
3 pts 5 Fu alone NR 7 pts previously untreated CR + PR 6/7 86% 5 pts previously treated 5 FU + RXT CR + PR 4/5 80%	short duration of the remission (6-18 mths) no significant difference between previously untreated pts and previously treated pts
CR 38/57 67% after completing all therapy modalities	
CR 11/23 48%	after a mean follow up of 44 mths 11/23 pts are L.FOD 23/26 pts completed therapy
	CHT + RXT 5 yrs survival rate 8/35 23% MST 22 mths
	CHT + RXT + S 5 yrs survival rate 7/15 44% MST 45 mths

all sites of head and neck tumours were involved, thus the results are not strictly comparable (Tables I and II).

Most randomized studies using concurrent combination therapy of 5Fu and radiotherapy as one treatment mode and radiotherapy alone as the other, show the combination mode as

having a substantial therapeutic advantage. The mean survival time differs in the studies of Ansfield et al. (1970), Shigematsu et al. (1971), Gollin et al. (1972), and is substantially higher for the combination treatment (Table III); only Fletcher et al. (1963) was unable to register differences in MST between the two treatment modes.

Wannenmacher et al. (1974) and Lo et al. (1976) recorded a clear superiority of the concurrent combination treatment (5Fu and RXT) in contrast to an historical control group in which radiotherapy only was used. Lo et al. (1976) were even able to record an increased survival period for oral cavity carcinoma (Table IV).

The large percentage of recurrences, even after complete response, results in poor survival after concurrent combination therapy with 5Fu and RXT. Early recurrence (within 1 year) was seen by Nitze et al. (1972) in 27% of patients, Wannenmacher et al. (1974) in 30%, Will et al. (1983) in 66% of patients, and Helpap et al. (1977) noted recurrence within 6 months in 44% of patients (Table IV).

In summary, intra-arterial administration of 5Fu has not demonstrated a superior effect over intravenous 5Fu in the clinical investigations reviewed.

Concurrent combination 5Fu and radiation therapy is superior to radiotherapy alone. Early recurrence occurs in more than one third of patients even after complete response.

4. Results of Concurrent Combined Administration of BLM and Radiotherapy.

The only study which has attempted by means of randomization to demonstrate superiority of intra-arterial over intravenous therapy is that of Shanta and Krishnamurthi (1977) which utilized concurrent administration of intra-arterial BLM with radiotherapy in one treatment mode, and intravenous BLM administration concurrently with radiotherapy in the other. No therapeutic advantage could be demonstrated for the intra-arterial administration of BLM (Table V). The objection to this study is however that the intra-arterial advantage was not optimally realized because the BLM was administered as a bolus, and local selective intra-arterial infusion (according to Bilder (1968)) was not utilized.

With intra-arterial administration Bleomycin did not cause lung complications, as it did with intravenous pulse administration (Shanta and Krishnamurthi, 1977). With intra-arterial administration, BLM comes into contact with the lung only after dilution in the systemic circulation. Using the intravenous pulse administration, the lungs are the first organ (a "target area") to be perfused with a high

(peak) concentration of BLM.
In randomized studies using BLM in the concurrent combination mode as compared to radiotherapy alone, Shanta and Krishnamurthi (1977), Kapstad (1979), Abe et al. (1978) and Phillips et al. (1980) (Table V) demonstrated a clear therapeutic advantage of the combination mode, as far as the complete response rate was concerned. Peres et al (1978), Cachin and Eschwege (1981) and Shah et al. (1981) (Table V) were unable to duplicate these findings in their studies. Shanta and Krishnamurthi (1977) and Kapstad (1979) recorded improved survival for the combination mode after a short-term follow-up. The other investigators were not able to show any difference in survival rates between the two treatment modes (Table V).

Berdal (1976) (Table VI) compared the short-term survival rate of his studies with an historical control group and found improved survival for the combination (BLM and RXT) over radiotherapy alone. Cruz et al. (1982) also made comparisons with an historical control group and found no difference in the long term survival rates of their concurrent combined (BLM and RXT) study and the historical radiotherapy control group.

If the complete response percentages in Table V are compared with Table VI (randomized versus non-randomized studies) the same large spread of results of therapy is evident, the complete response being 37 - 84% in Table V and 33 - 92% in Table VI. For radiotherapy alone, in the randomized studies of Table V the complete response is also extremely variable (between 15 - 68%). This variability is again associated with considerable heterogeneity of the patient material, due to difference in staging, difference in localization, and difference in administration mode (intra-arterial versus intravenous pulse). Despite attempts to group the patient material according to identical staging, localization and administration mode, the variability in the results of therapy is still great.

The importance of the localization of the primary tumour in determining the prognosis can be seen from the figures of Berdal (1976), who recorded the highest percentage of complete responses for oral cavity carcinomas and the lowest percentage of complete responses for tonsil carcinomas (Table VII).

Matsumura and Motomura (1973), Tanaka et al. (1976) and Suzuki (1976) treated antrum carcinomas and administered half of the normally used total dosage for concurrent combination treatment (BLM and RXT) followed by surgery. Even after treatment with half of the usual total dose, Matsamura and Motomura (1973) and Tanaka et al. (1976) found complete responses in 40% and 33% respectively of their patients. In 8 of the 11 histologically examined resection specimens of

TABLE III RESULTS OF CONCURRENT COMBINED 5 FU ADMINISTRATION AND RADIOTHERAPY (RANDOMIZED STUDIES)

AUTHOR	ADMINISTRATION ROUTE	STAGING SITE NUMBER	DOSE SCHEDULE
Fletcher (1963)	i.v. cont.inf.	all sites all stages 19	5 Fu i.v. RXT 6000 r (TD) (if RXT alone TD 8000 r)
Ansfield (1970)	i.v. pulse	advanced disease all sites 134	5 Fu 10 mg/kg/day on days 1 to 3 5 mg/kg/day on day 4 5 mg/kg/day 3 x/wk RXT 200 r 5 fx/wk TD 6500r 6.5 wks
Gollin (1972)	i.v. pulse	advanced disease all sites 155	CHT 5 Fu 10 mg/kg/day on day 1 to 3 5 mg/kg/day on day 4 5 mg/kg/day 3 x/wk RXT 200 r 5 fx/wk TD 6500 r in 6.5 wks sometimes followed by S
Shigematsu (1971)	i.a. cont.inf.	T1-4, N0, M0 max.sinus CA 47	5 Fu 10 mg/kg/day RXT 200 r fx/wk TD 8000 r

TABLE IV RESULTS OF CONCURRENT COMBINED INTRAVENOUS 5 FU ADMINISTRATION AND RADIOTHERAPY

AUTHOR	ADMINISTRATION ROUTE	STAGING SITE NUMBER	DOSE SCHEDULE
Hall (1961)	i.v.	advanced disease H and N CA 15	5 Fu 15 mg/kg/day on day 1 to 3 7½ mg/kg/day on day 4 7½ mg/kg/day 3 x/wk RXT 2000 r TD

RANDOMIZATION	REMARKS SURVIVAL
CHT + RXT 8 RXT 11	no differences in MST between the two groups no differences in the CR + PR rates in the two groups poor results with T4 tongue, floor of mouth CA
CHT + RXT 68	MST 27 mths. survival rate after 18 mths 52.7 % 3 yrs 44.5 % 5 yrs 36 %
RXT 66	MST 14 mths. survival rate after 18 mths 40.6% 3 yrs 18.8% 5 yrs 15 %
CHT + RXT 76	MST 28.3 mths MST T3 31.7 mths MST T4 20 mths MST oral cavity CA 32.3 mths
RXT 79	MST 19.8 mths. MST T3 16.8 mths MST T4 15 mths MST oral cavity CA 16.6 mths

	1 yr	2 yr	NED 1 yr	NED 2 yr
CHT + RXT + S 17	76%	55%	47%	37%
RXT + S 30	67%	58%	23%	29%

short term survival rate is better

RESPONSE RATE		REMARKS SURVIVAL
CR	PR	
CR + PR 10/15 66%		

Nitze (1972)	i.v. intermitt. cont.inf.	advanced disease 18	5 Fu 18-20 hrs after 8 hrs interruption RXT 500 r 2 fx/wk TD 6000 r
Wannenmacher (1974)	i.v. intermitt. cont.inf.	stage III + IV 46	5 Fu 1 gr 12 hrs 2 x/wk RXT 500 r 2 x/wk TD 6000 r previously untreated TD 4000 r previously treated
Lo (1976)	i.v. cont.inf.	? 33	5 Fu 10 mg/kg/day on day 1 to 3 5 mg/kg/day on day 4 5 mg/kg/day 3 x/wk RXT 200 r 5 fx/wk hist.contr.group RXT alone 13
Helpap (1979)	i.v. intermitt. cont.inf. t = t G_1 + 2 hrs	advanced disease all sites 9	5 Fu 500 mg RXT 150 r 5 fx/wk TD 6000 r
Esser (1979)	i.v. intermitt. cont.inf. 12 hrs	stage III + IV 29	5 Fu 1 gr 2 x/wk RXT after 12 hrs 500 r 2 fx/wk TD 5000 r
Dietz (1979)	i.v. intermitt. cont.inf. 12 hrs	inoperable advanced disease oropharynx + tongue 20 pts nasopharynx + nose 6 pts	5 Fu 1 gr 2 x/wk RXT after 8 hrs 400 r 2 fx/wk TD 4800 r
Will (1983)	i.v. intermitt. cont.inf. 12 hrs	non-resectable all sites 13	5 Fu 1 gr 2 x/wk RXT after 8 hrs rest 500 r 2 fx/wk TD 4500 r

CR	
11/18 61%	A.NED 7/15 46% (short follow up) of the CR's 3/11 27% showed quick relapse
	MST of all pts in the study 5.5 mths MST of the 19previously untreated pts with RXT 12.8 mths A.NED 27/40 68% (short term follow up) but 30% showed a quick relapse survival rates of historical controls with RXT alone was worse than those treated with combination therapy
16/33 48%	A.NED after a short term follow up 11/33 33% 2 yr survival rate 54%
8/33 24%	A.NED 5/33 15% (short term follow up) 2 yr survival rate 18% oral cavity CA gave significantly better results
9/9 100%	3.5 yr follow up A.NED 3/9 33% REC 4/9 44% 2/9 22% failed to complete therapy due to toxic side effects
23/29 81%	MST 16.2 mths pulm.metas. 37.9%
CR + PR (deutliche remission) 14/20 70%	5 yr survival rate 9% CR's also showed rapid recurrence
3/13 23%	REC rate 66% (follow up $<$ 12 mths)

TABLE V RESULTS OF CONCURRENT COMBINED BLM ADMINISTRATION AND RADIOTHERAPY (RANDOMIZED STUDIES)

AUTHOR	ADMINISTRATION ROUTE	STAGING SITE NUMBER	DOSE SCHEDULE
Shanta (1977)	i.a. pulse	oral cavity T3 T4 advanced disease 116	BLM 10-15 mg i.a. pulse + RXT RXT 200 r 5 fx/wk TD 5500-6000 r BLM 10-15 mg i.v. pulse + RXT RXT 200 r 5 fx/wk TD 5500-6000 r
Peres (1978)	i.m.	all sites all stages 38	BLM 15 mg/day every 3rd day for 10 wks TD 300 mg + RXT RXT 250 r 5 fx/wk TD 5000-7000 r
Kapstad (1979)	i.m.	all sites stages III + IV 29	BLM 15 mg 3 x/wk i.m. 1 hr before RXT RXT TD 3000 r interruption after 1500 r RXT + placebo till 3000 r followed by 7000 r or S
Abe (1978)	i.v. pulse	all stages oral CA 67	BLM 15 mg before RXT TD 3000 r followed by S RXT 200 r 5 fx/wk followed by S
Phillips (1980)	i.v. pulse	stages III + IV non-resectable inoperable H and N CA	BLM 5 U/day 2 x/wk + RXT 180-200 r 5 fx/wk TD 7000 r. followed by 15 mg BLM 1 x/wk for 16 wks RXT 180-200 r 5 fx/wk TD 7000 r

RANDOMIZATION	RESPONSE RATE		REMARKS SURVIVAL
	CR	PR	
i.a. BLM + RXT 42	33/42 78.5%		DFS 2 yr 4 yr RXT 11% 9% RXT + BLM 66% 50%
RXT 32	7/32 21.8%		
i.v. BLM + RXT 22	17/22 77.3%		
RXT 20	4/20 20%		i.a. no pulmonary side effects i.v. pulse gave pulmonary toxicity
CHT + RXT 19	7/19 37%	2/19 11%	A.NED 5/19 26% (follow up $>$ 6 mths)
RXT 19			
CHT + RXT 15	CR (controlled pts.) 13/15 87%		better short term survival rate ($<$ 2 yrs) for the combination therapy
RXT + placebo 14	9/14 64%		
CHT + RXT + S 33	15/33 44% after using half the combination therapy as initial treatment		severe mucositis 78%
RXT + S 34	5/34 15% after 3000 r as initial treatment		severe mucositis 15%
CHT + RXT + S 17	6/13 84.6% (13/17 completed therapy)		MST 12 mths
RXT 12	3/7 62.5% (7/12 completed therapy)		MST 12 mths

AUTHOR	ADMINISTRATION ROUTE	STAGING SITE NUMBER	DOSE SCHEDULE
Cachin (1981)	i.v. pulse i.m.	all sites all stages 186	BLM 15 mg/day i.v. pulse or 15 mg/day i.m. 2 x/wk 2 hrs before RXT TD 150 mg in 5 wks RXT 200 r 5 fx/wk TD 7000 r
Shah (1981)	i.v. pulse	all sites stages III + IV 59	BLM 15 mg/day every 2nd day followed by RXT within 30 min RXT 200 r 5 fx/wk

TABLE VI RESULTS OF CONCURRENT COMBINED BLM ADMINISTRATION AND RADIOTHERAPY

AUTHOR	ADMINISTRATION ROUTE	STAGING SITE NUMBER	DOSE SCHEDULE
Matsumura (1973)	i.m. 10 pts i.v.pulse 8 pts i.m. 3 pts	advanced sinus CA 22	BLM 15-30 mg i.v. pulse or 15-30 mg i.m. or 15-30 mg i.a. 2-3 x/wk RXT 200 r 5 fx/wk TD 3000 r followed by S followed by RXT TD 7000 r
Wannenmacher (1975)	i.v. pulse	stages III + IV 18	BLM 7.5-15 mg for 2 days 6 hrs after last dose RXT 500 r 2 fx/wk TD 4000-5000 r
Tanaka (1976)	i.v. pulse	all sites T2 T3 T4 39	BLM 15 mg 2 x/wk RXT 200 r 5 fx/wk TD 3000 r after half the combination therapy followed by S

RANDOMIZATION	RESPONSE RATE		REMARKS SURVIVAL
	CR	PR	
CHT + RXT 99	66/99 67% lymphnodes:	62%	there are no significant differences in the 3 and 5 yr survival rates of both groups
RXT 87	59/87 68% lymphnodes:	49%	
CHT + RXT 23	11/23 48%		MST responders 7 mths
RXT 36	18/36 50%		MST responders 6 mths.

RESPONSE RATE		REMARKS SURVIVAL
CR	PR	
9/22 40%		FOD > 1 yr 7/22 32% REC < 6 mths 13/22 59% after using half the concurrent combination therapy as initial treatment 1/3 of the patients are FOD at short term follow up
11/18 61%		survival rate CR's > 12 mths 45% of the CR's were FOD (short term follow up) there was no correlation between tumor regression and G_2+M increase during therapy
13/39 33%		survival rate > 2 yrs 30/39 77% no tumour histologically evident in 8/11 resection specimens examined

AUTHOR	ADMINISTRATION ROUTE	STAGING SITE NUMBER	DOSE SCHEDULE
Suzuki (1976)	i.v. pulse	sinus CA 29 sinus CA 115 hist. contr.group	CHT + RXT (Tanaka) followed by S RXT and/or S alone
de la Garza (1976)	i.v. pulse	all sites all stages 20	BLM 15 mg 3 x/wk RXT 200 r 5 fx/wk TD 4000-6000 r
Berdal (1976)	i.m.	all sites all stages 27	BLM 1st wk 15 mg/day 6 x/wk 1 hr before RXT 2nd wk 15 mg/day 3 x/wk 3rd wk 15 mg/day 3 x/wk 1 hr before RXT RXT TD 4300 r
Greiner (1979)	i.m.	all sites T3 T4 5	BLM 0.1 mg/kg/day (max. 5 mg/day) 1 hr before RXT 120-200 r 5-6 fx/wk
	up front cont.inf. 24 hrs. 8 days	all sites T3 T4 15	BLM 7.5 mg/day cont.inf. 8 days followed by RXT TD 6000 r
Rijgård (1979)	i.m.	all sites all stages 33	BLM 10-15 mg 2-3 x/wk 1-1½ hrs before RXT 160-200 r 5 fx/wk followed by S if no CR occurs
Seagren (1979)	i.m.	all sites stages III + IV 19	BLM 15 mg 15 min. before RXT 180 5 fx/wk TD 5000 r followed by RXT TD 7000 r and/or S
Silverberg (1980)	i.v. pulse	stages III + IV non- resectable inoperable 42	BLM 19 pts 15 mg 2 x/wk 18 pts 2 mg 2 x/wk 2 pts 2 mg 2 x/wk RXT 180 r 5 fx/wk TD 7000 r tumour TD 5000 r neck

RESPONSE RATE		REMARKS SURVIVAL
CR	PR	
		5 yr survival rate 62%
		5 yr survival rate 33%
CR +(PR) (exellent response) 5/20 25%		NED 5/20 25% (9-14 mth follow up)
oral cavity CA 19/34 56% max. + sinus CA 3/15 20% tonsil CA 2/11 18%		REC 6/19 32% FOD 68% (3-10 mth follow up) short term survival (< 2 yrs) of the combination therapy better than a historical control group using RXT alone, long term survival rate (> 3 yrs) was no better for the combination therapy
oral cavity mesopharynx CA 4/5 80%		after 33 mths 11% of the CR's NED
oral cavity mesopharynx CA 6/15 40%		no correlation between the degree of mucositis and the CR
15/33 45% after CHT + RXT + S 21/33 63%		FOD 21/33 63% (short term follow up) 66% severe mucositis
11/19 58% CR + PR 89%		DFS > 1 yr 58%
23/43 52%	18/42 43%	survival rate > 1 yr 13/23 CR 57% recurrence rate < 1 yr 10/23 43% 5 mg 2 x/wk gave dramatically less mucositis

AUTHOR	ADMINISTRATION ROUTE	STAGING SITE NUMBER	DOSE SCHEDULE
Lindholm (1982)	i.m.	*stages* III + IV 7	BLM 2½ mg/day 1–2 hrs before RXT 2 x 100 r day
Cruz (1982)	i.m.	stages III + IV *all sites* 33	BLM hydroxyureum + 2–5 U BLM 3 x/wk RXT 200 r 5 fx/wk TD 6000 r tumor TD 5000 r neck sometimes split course because of mucositis
RESULTS OF CONCURRENT COMBINED INTRAVENOUS CISPLATINUM ADMINISTRATION AND RADIOTHERAPY			
Slotman 1986	i.v.	all sites non-resectable T3 T4 CA 18	20 mg/m^2 Cis-pl. on days 1–4 and 21–24 before RXT 180 r 5 fx/wk/day TD 4500 r followed by S (12/18)

patients who showed complete response, no tumour was found (Tanaka et al., 1976).

In multi-modality treatment schedules, for example concurrent combined Bleomycin and radiotherapy followed by surgery, significantly better survival rates are found than for radiotherapy followed by surgery. The 5 year survival figures rose from 33% to 62% in Suzuki's (1976) study. Rygård and Hansen (1979) also recorded a similar rise but for a short-term follow-up.

Greiner et al. (1979) (Table VI) showed in his study that the percentage of complete responses for the concurrent combination treatment (BLM and RXT) is higher (80%), than for induction chemotherapy treatment with Bleomycin followed by radiotherapy (40%).

The local toxicity is clearly greater in combination treatment and some investigators do not find the "limited" therapy advantage outweighs the percentage of patients unable to complete the treatment because of toxicity (Abe et al., 1978; Cachin and Eschwege, 1981). Rygård and Hansen (1979) recorded serious mucositis in 66% of patients during

RESPONSE RATE		REMARKS SURVIVAL
CR	PR	
7/7 100%		survival rate $>$ 20 mths 86% NED
16/34 37%	17/39 39%	long term survival rates not different from historical control group (16%) group (16%)
13/18 72% CR + PR 16/18 89%		one therapy-related death 5/10 resected tumours with CR were histologically negative for tumour

combination treatment.

In summary:

1. Clinical research has not demonstrated that there is a superior effectiveness of intra-arterial bolus administration over intravenous administration of BLM during combination therapy.
2. Randomized investigations with concurrent Bleomycin and radiotherapy versus radiotherapy alone, show considerably varying results. However, in short-term follow-up studies (Shanta and Krishnamurthi, 1977; Kapstad, 1979) better survival percentages were registered for combination therapy than for radiotherapy alone.
3. For the achievement of a complete response, concurrent combination therapy (BLM and RXT) is better than induction chemotherapy with BLM followed by radiotherapy (Greiner et al., 1979).
4. Combination treatment compared to radiotherapy alone, is clearly inferior as far as local toxicity is concerned (Cachin and Eschwege, 1981; Abe et al., 1978).

TABLE VII RESULTS OF CONCURRENT COMBINED ADMINISTRATION OF A MULTIDRUG REGIME AND RADIOTHERAPY

AUTHOR	ADMINISTRATION ROUTE	STAGING SITE NUMBER	DOSE SCHEDULE
Fu (1979)	i.v.	stages III + IV inoperable non-resectable Karnofsky > 60	cyclophosph 750 mg/m^2 1 x/wk Vi 1.4 mg m^2 1 x/wk BLM 15 mg 2 x/wk + RXT 180 r 5 fx/wk total 3 cycles of 2 wks with 2 wks interruption
Smith (1981)	i.v.	stage IV 24 stage III 11 tot 36	BLM 4.5 U/m^2 2 x/wk ADR 7 mg/m^2 1 x/wk 5 Fu 110 mg m^2 2 x/wk 1 hr before RXT RXT 200 r 6 fx/wk followed by S only
Ammon (1981)	i.v. pulse	advanced H and N tumours previously treated pts 17 plav.cell. CA 2 melanomas 1 osteosarcoma 3 adeno CA 28	ADR 20 mg after 12 hrs Vi. 0.5 mg after 12 hrs RXT 300-400 r 2 fx/wk for 10 wks
Forastiere (1982)	i.v.	advanced disease 6	BLM 15 mg 3 x/wk RXT 180-200 r 5 fx/wk for 2 wks 2 wks. interruption + Cis.plat. followed by 2 wks combination therapy
Seagren (1982)	i.v. i.m.	advanced disease T3 T4 N2 N3 24	Cyclophosph 1 gr/m^2 i.v. 1st day BLM 15 Units/day i.m. 2nd 4th 9th 11th day RXT 180 r 5 fx/wk for 2 wks 2 wks interruption followed by the next cycle TD 5400 r followed by S

RESPONSE RATE		REMARKS SURVIVAL
CR	PR	
3 mth follow up 8/15 53%		short term follow up (5-24 mths) CR's → NED 7/15 46% death during therapy 5/15 33% 3/15 pneumonia 1/15 tox.shock 1/15 CVA duration of PR very short < 8 mths
20/30 66% CR + PR 29/30 79%		A.NED 12/30 40% (6-27 mth follow up) all stage IV pts died during follow up 17% failed to complete therapy due to toxicity
8/28 29%		all pts had previous treatment poor prognostic group
4/6 66%		survival rate after 1 yr: A.NED 1/6 17% CR's A.NED 25% 50% did not complete therapy due to high toxicity
after CHT + RXT 14/24 54%		5 mth follow up: 68% showed local recurrence 63% showed distant metatstasis N + pts showed a worse prognosis than T4-N0 pts.
after CHT + RXT + S 16/24 67%		

AUTHOR	ADMINISTRATION ROUTE	STAGING SITE NUMBER	DOSE SCHEDULE
Ringborg (1982)	i.v. i.m.	all sites all stages 30 non resectable 3 inoperable 33	BLM 25 mg i.m. Cyclophosph 100 mg i.v. 3 Fu 125 i.v. 1 hr before RXT 2 x 100 r./day 5 fx/wk with 21 pts 200 r 5 fx/wk with 12 pts in 5 cycles 2 wks interruption after each cycle
Fairman (1982)	i.v. i.m.	advanced H and N CA previously untreated pts 11	ADR 60 mg/m^2 i.v. 5 min. before hyperthermia for during 4-6 wks, followed by BLM 15 mg 3 x/wk i.m. 30 min. before hyperthermia RXT 4 hrs before CHT + hyperthermie followed by S 1 pts
Kreidler (1983)	i.a. intermitt. cont.inf. 12 hrs local selective	advanced disease non-resectable H and N CA 14	ADR 6-12 mg 2 x/wk BLM 7½-15 mg 2 x/wk RXT 300 r 3 fx/wk within 6 hrs. after inf. TD 4000 r S 2 pts with REC neck diss. at N + neck before CHT + RXT
Szepesi (1984)	i.a. inf. 2 hrs	inoperable H and N CA 66	MTX 15 mg every night BLM 15 mg every morning 4 hrs after BLM inf. followed by RXT TD 6000-6500 cGy
Hollman (1984)	i.a. inf. 2 hrs	inoperable H and N CA 103	"

RESPONSE RATE		REMARKS SURVIVAL
CR	PR	
15/33 46% correlation with T-stage T2 9/14 60% T3 4/14 36%	12/33 36%	survival rates are significantly better for CR's than NR's 58% of the CR's are alive > 2 yrs at 3 yr follow up there was no better survival for the CR's compared with the other pts in the study
7/7 100%		follow up > 18 mths: NED 5/5 71% REC 2/7 29% after 12 and 16 mths therapy-related severe toxicity 4/11 pts died during therapy (36%)
14/14 100%		19-56 mths follow up: A.NED 9/14 66% 3/14 21% DWD FOD after salvage S 2/14
17%	48%	CR average survival 82 mths PR average survival 9 mths CR's 5 yr survival rate 45% PR's 4 yr survival rate 3%
"	"	correlation between survival and: 1. age 2. previously untreated 3. no evidence of bone invasion 4. BLM dosage > 250 mg 5. type of radiation 6. radiation dose > 4500 cGy

TABLE VIII RESULTS OF SEQUENTIAL CHEMOTHERAPY AND RADIOTHERAPY
(Split course radiotherapy - chemotherapy during the interruptions)

AUTHOR	ADMINISTRATION ROUTE	STAGING SITE NUMBER	DOSE SCHEDULE
Malaker (1980)	i.v.	stages III + IV all sites 29 oral cavity 5 tonsil 4 larynx 10	1. Price-Hill regime 2. 2000 r 200 r 5 fx/wk for 2 wks 3. Price-Hill regime 4. 2000 r etc. TD RXT 6000-6600 r and 3 cycles CHT
Clifford (1979)	MTX i.v. intermitt. cont.inf. BLM i.m. Vi. i.v. pulse	stages II + IV all T all sites	wk. 1, 4, 7, 10 CHT Vi. 2 mg i.v. pulse after 6 hrs BLM 30 mg i.m. followed by MTX 50 mg i.v.cont.inf. + leucov.resc. 9 mg 4/day RXT wk 2, 3, 5, 6, 8, 9 180-200 r 5 fx/wk TD 2000 r in 2 wks followed by S for PR's hist.contr.group RXT alone and/or S
O'Connor (1980)	MTX i.v. intermitt. cont.inf. Vi. i.v. pulse	all sites all stages H and N CA 92 pts	sequential VBM regime + RXT sometimes followed by S (O'Connor 1983) hist.contr.group RXT alone and/or S 92 pts
O'Connor (1983)	"	stages III + IV all sites 198 oral cavity CA 45 pts	sequential VBM regime + RXT, sometimes followed by S VBM regime (Clifford) with a higher dose of MTX 200 mg in 24 hrs + leucov.resc 15 mg i.v. + 15 mg i.m. 4 x/day RXT TD 6000-6600 r

RESPONSE RATE		REMARKS SURVIVAL
CR	PR	
23/29 79% N2 neck 11/14 79%		3/29 10% died during therapy, due to pneunomia abcess CVA sepsis 5/29 pts. developed lung metastases during the follow up period
84/102 82% CR + PR = 92/102 91%		A.NED 56.2 mths
CR + PR 54/92 59% CR (cure)26/92 28%		A.NED 21.9 mths

survival rate after

12 wks	30 mths	50 mths	60 mths
74.5	56.5	48.5	48.5
64	35	27.5	26

died during therapy
10/92 15%

30 mth follow up: REC 8/37
NED 29/37 78%
there is a correlation between N+and:
1. poor survival rate
2. increased recurrence rate
3. death during therapy
4. occurrence of distant metastases during follow up

AUTHOR	ADMINISTRATION ROUTE	STAGING SITE NUMBER	DOSE SCHEDULE
Bardual (1982)	"	all sites all stages 30 pts 14 oral cavity oropharynx lip 16 larynx	sequential VBM regime + RXT (Clifford) sometimes followed by S

5. Results of Concurrent Combined Administration of Multidrug Chemotherapy and Radiotherapy.

When the complete responses of Table VII are compared,

		CR %	Combination chemotherapy used
Fu et al.	(1979a.)	53	cyclo ph Vi BLM
Smith et al.	(1981)	66	ADR BLM
Forastiere et al.	(1982)	66	Cis pl BLM
Seagren et al.	(1982)	54	cyclo ph BLM
Ringborg et al.	(1982)	46	cyclo ph 5Fu BLM
Fairman	(1982)	100	ADR BLM
Kreidler & Petzel	(1983)	100	ADR BLM
Szepesi et al.	(1985)	17	MTX BLM

it is obvious that with concurrent combination treatment with polychemotherapy and radiotherapy few or no more patients achieve complete response than with concurrent BLM and radiotherapy. The two studies which are clearly at variance, are those of Fairman (1982) and Kreidler and Petzel (1983). These studies contain a small number of patients, but record 100% complete response after combination treatment with ADR and BLM concurrently with radiotherapy. The results of the two studies differ however from the other investigators; Fairman (1982) applied concurrent hyperthermia with intravenous or intramuscular chemotherapy 4 hours after radiotherapy, and Kreidler and Petzel (1983) administered chemotherapy intra-arterially via local selective infusion concurrently with radiotherapy. The percentage of short-term survival (from 14 - 56 months) was 78.6%.

Seagren et al. (1982) recorded a high percentage (68%) of local and regional recurrence within 5 months in patients who initially had a complete response. It also appeared that in 63% of patients with local recurrence, a distant metastasis had occurred. A worse prognosis for an N+ staging was also shown in his study.

Fu et al. (1979a.) saw a high percentage (46%) survival after

RESPONSE RATE		REMARKS SURVIVAL
CR	PR	
12/30 40%	15/30 50%	no correlation between tumour response and staging
CR + PR 90%		there is a correlation between tumour response and survival rate

short-term follow-up for patients who had achieved complete response (biological selection). Ringborg et al. (1982) recorded 58% survival after a short follow-up in complete responders. Biological selection was demonstrated by Ringborg et al. (1982) who found a correlation between short-term survival and the extent of response. The short-term survival was shown to be significantly better for the complete responders than for the non-responders. The percentage of complete responders was also shown to be dependent on the T stage of the tumour. There was a higher percentage of small tumours than large tumours in complete remission.

In summary, it can be said with regard to multidrug regimes administered concurrently with radiotherapy, that:

1. The results, stated as percentages, for complete response are not significantly better than for treatment with BLM concurrently with radiotherapy.
2. The combination treatment carried out with concurrent chemotherapy, hyperthermia and radiotherapy clearly increases the complete response rate (Fairman, 1982).
3. The combination treatment carried out with intra-arterial local selective infusion concurrently with radiotherapy results in an extremely high complete response rate (Kreidler and Petzel, 1'83).
4. The percentage recurrence is high, even after the achievement of a complete response (Seagren et al., 1982). For this reason, research into the determination of biological prognostic parameters relating to possible cure occurring in concurrent combination treatment is indicated (this dissertation).

6. Results of Sequential Treatment with Multidrug Chemtherapy and Radiotherapy.

In order to reduce the increased local toxicity of concurrent combination treatment, some investigators have used an alternating (sequential) treatment. Inspired by the "split course" radiotherapy regime, chemotherapy is administered during the intervals before and after radiotherapy.

Clifford (1979) and O'Connor et al. (1980 a,b, 1983) used a

VBM chemotherapy regime (Table VIII). O'Connor's study (1980a.b., 1983), involving oral cavity carcinomas, showed that 37 of 45 (82%) achieved complete response. The short-term survival percentage (after 30 months) was good (29 of 37 (78%)). Clifford (1979) similarly recorded 84 of 102 (82%) complete responders with an average tumour-free survival of 56.2 months. Both investigators used historical control groups with only radiotherapeutic and/or surgical treatment as comparison material for their findings. Both Clifford (1979) and O'Connor et al. (1980a.b., 1983) saw significantly better results in their patients who underwent "sequential" treatment, as compared to the historical control group (Table VIII).

Bardual et al. (1982) also used the VBM regime with sequential radiotherapy, but recorded a clearly lower complete response rate (40%). Bardual et al. (1982) showed biological selection and the survival rate was seen to correlate with the extent of response to sequential therapy.

Malaker et al. (1980) using a Price-Hill chemotherapy regime (Table VIII) were able, as in the VBM regime of O'Connor et al. (1980, 1982) and Clifford (1979), to achieve a high complete response rate (79%). The response of large cervical lymph node metastases was significantly less (21%) than the response for primary tumours in the research by Malaker et al. (1980).

Pearlman et al. (1982) randomized Stage III and IV tumours and non-resectable cervical lymph node metastases N_{3a} (AJC 1978), using a CBM chemotherapy regime sequentially with radiotherapy in the one treatment mode, and standard therapy (radiotherapy and/or surgery) in the other mode, and found an advantage for the sequential mode for the higher T and N stages in the average survival, after a follow-up of 14 - 47 months.

In summary, with regard to sequential treatment with multi-drug chemotherapy and radiotherapy, it can be stated that:

1. The sequential treatment results in at least as many complete responses as the concurrent combination treatment, with the exceptions of a) the combination treatment concurrent with hyperthermia (Fairman, 1982), and b) the combination treatment with intra-arterial local selective infusion (Kreidler and Petzel, 1983).
2. In comparison with historical control groups, the combination treatment is superior to the standard treatment(s) (radiotherapy and/or surgery), with regard to both complete response rate and survival rate (Clifford, 1979; O'Connor et al., 1980a,b, 1983;). In a randomized study, Pearlman et al. (1982) demonstrated an increased survival for sequential combination treatment for T_4 and N_{3a} staged (AJC) tumours.

3. Local toxicity occurring in sequential combination therapy is less than in concurrent combined therapy. However, the mortality of sequential combination therapy is approximately the same as for concurrent combined therapy. Malaker et al. (1980) recorded a 10% mortality rate during treatment, and O'Connor et al., (1980a,b.) 15%.

CHAPTER IX

Local Selective Intra-arterial Infusion

1. Acknowledgement.

The hypothetical pharmacokinetic two compartment model used in these studies was devised with the help of R.G.F.Knol, M.Sc.

1.1. A Hypothetical Two Compartment Model for Investigation of the Possible Superior Effectiveness of Intra-arterial Infusion over Intravenous Infusion.

The conclusions of this hypothetical model are based on mathematical calculations which can be found in Appendix I.

a. The duration (ΔT) of the infusion does not influence the superior effectiveness of intra-arterial infusion.

b. The total dose (TD) has no effect on the superior effectiveness of intra-arterial infusion.

c. The concentration in the target area is always higher during intra-arterial infusion than after it, when the concentration is due only to recirculation.

The final benefits of intra-arterial administration can be calculated by computing the total amount of cytostatic agents taken up in the target area before and after the end of infusion for intra-arterial and intravenous administration routes respectively. From mathematical derivation of the above expression, follows the equation:

I.a. infusion superiority = $R_d^{\infty} = 1 + (\frac{1}{a} - 1)\, d_s$
(see Appendix I)

d. The concentration advantage is to a great extent dependent on the flow in the target area artery (the flow in the area of the catheter tip = a ; when a decreases the Rd increases.

e. The superior effectiveness of intra-arterial infusion (R_d^{∞}) is also determined by the absorption-excretion-detoxification capacity of the cytostatic agent in the rest of the body (ds). (As ds increases, Rd also increases).

f. The superior effectiveness of intra-arterial infusion (R_d^{∞}) appears to be independent of the absorption capacity of the target area.

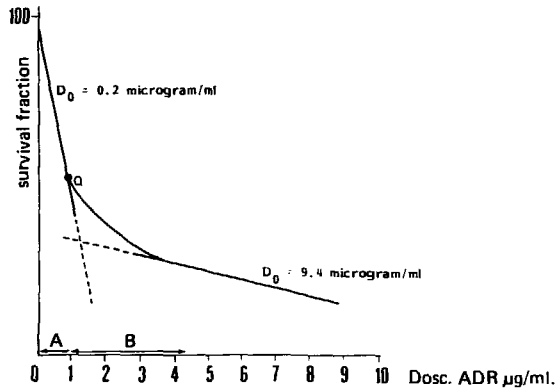
1.2. The Use of Different Drug Concentrations for Maximization of the Superior Effectiveness of Intra-arterial Infusion.

The dose-survival curve for the drugs Adriamycin and

Bleomycin is always biphasic with a small D_0 for the low concentrations and a larger D_0 for the high concentrations (Fig. 19). This was demonstrated for Adriamycin by Barranco et al. (1973, 1974) and for Bleomycin by Terasima (1976a), Urano et al. (1973), Barranco and Humphrey (1976) and Olah et al. (1978).

In the low concentration range (A in Fig. 19), the effectiveness on the tumour is thus greater than for concentrations in the high concentration range (B in Fig. 19).

Fig. 19



Typical survival curve of a cell line after incubation with Adriamycin (Barranco, 1973).
A = low concentration range
B = high concentration range

From the conclusions stated earlier, it can be seen that the superior effectiveness of intra-arterial infusion is not influenced by infusion time (ΔT). Therefore, the infusion duration which maximally utilizes the advantage in the steep part of the dose-effect curve, can be chosen. The low concentration range associated with a low D_0 occurs with continuous intra-arterial infusion. Concentrations associated with a higher D_0 are attained at peak levels during and just after bolus administration. It is further well known that incubation time is inversely related to the survival of incubated cell lines until a steady state is attained (Terasima, 1976).*

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* After all the predisposition sites have been occupied in the DNA helix by Adriamycin or Bleomycin, the binding capacity is diminished and a steady state is attained. Steady state conditions are attained after 6 - 10 hours in in vitro studies with Adriamycin (Noël et al., 1981).

The systemic toxicity of a cytostatic agent is determined by the total dose which is taken up in the body outside the target area. Theoretically, a large extraction fraction by the tumour (di) should result in a lower total dose in the rest of the body. In practice, the di is so low in comparison to the total dose (TD) that there is no likelihood of a reduced systemic toxicity. Only liver perfusion via the hepatic artery with a cytostatic agent which is largely detoxified (high organ extraction capacity), results in a reduced systemic toxicity using this administration route.

1.3. Conclusions.

a. The duration of the infusion (ΔT) does not influence the superior effectiveness of intra-arterial infusion.
b. There is equally no influence of the total dosage (TD) on the superior effectiveness of intra-arterial infusion.
Both (a and b) can thus be chosen in order that optimalization of other conditions can be attained, such as:
c. Termination of the infusion after the attainment of a steady state.
d. The achievement of the highest possible concentration in the (low) favourable concentration range of the dose-survival curve of the cytostatic agent used (point Q in Fig. 19)
e. Furthermore, reduction of the target area (Bilder, 1968, 1969; Bilder et al. 1970 and 1974) is of great importance for superior effectiveness of intra-arterial infusion.
When c., d. and e. are carried out (at a constant total dose (TD)), the greatest therapeutic effect can be expected from intermittent (c), continuous (d), local selective (e) intra-arterial infusion.

2. Clinical Observations Supporting the Superiorty of Intra-arterial over Intravenous Infusion.

2.1. Oral Mucous Membrane Toxicity after Intra-arterial Infusion and after Systemic Recirculation.

In our own patient material, all treated patients clearly showed increased mucosal toxicity in the intra-arterially perfused area as compared to the mucosa perfused by recirculation, Bierman et al. (1951). Sullivan et al. (1953), Stephens (1983) and Kreidler and Petzel (1983) reached the same conclusion. After three weeks of combination treatment, all patients showed on the intra-arterially infused side, precisely within the perfused area (target area), mucositis of increased severity: (Grade III, Appendix III.) confluent exudative mucositis tending to ulcerative areas. A milder form (Grade I) of mucositis (erythema) was evident on the contralateral side which was perfused by recirculation.

2.2. Histopathological Observations in a Tumour treated in part by Local Selective Intra-arterial Infusion and in part by Recirculation.

In a left-sided tumour which was treated by way of local selective intra-arterial infusion, invasion over the mid-line was minimal. The tumour was thus treated by single-sided intra-arterial infusion with drugs concurrently with radiotherapy. During the therapy complete response of the extensive tumour occurred. After approximately 4 months an ulcer was detected paramedially on the right, which within 2 weeks caused a oro-nasal fistula. Recurrence in the contralateral right side led to salvage surgery of the whole maxilla, velum, nasopharynx, and mesopharynx (Case 11, Part II, Ch. X, 3.). The location of extension of the primary tumour over the mid-line was thus the area of local recurrrence. On histopathological examination of the resection specimen, the initially affected area (the left alveolar process and palate) no longer contained living tumour tissue, only keratin pearls and atrophy of the mucoperiosteum. The area of the median suture did contain vital tumour tissue (Fig. 20). This was the area which was treated with chemotherapeutic drugs by recirculation.

2.3. Disulphine Blue Staining of the Target Area after using Local Selective Intra-arterial Infusion and after Systemic Recirculation.

Intra-arterial infusion of disulphine blue results in more protracted persistence of staining and a higher intensity (concentration) of staining which is more rapidly evident in the target area than does systemic (via recirculation) perfusion. Stephens (1983) and Kreidler and Petzel (1983) have confirmed our observations.

The bright blue colour is, after a short time, visible in the target area directly after intra-arterial infusion (Part II, Fig. 5.). The staining after recirculation after a longer time, is diffusely uniform in colour with obviously reduced intensity and differs from the staining in arterially perfused areas which is sharply bordered with random vessel branches and sharply defined up to the mid-line.

3. Experimental Studies Comparing Intra-arterial and Intravenous Administration of Cytostatic Agents.

Didolkar et al. (1978) compared different administration modes for Adriamycin with each other. In an experiment in which isolated perfusion (extracorporal), intra-arterial infusion, and intravenous infusion was used, they recorded the highest concentration in the isolated perfusion method. The intra-arterial administration resulted in a higher (by a

Fig. 20

a-bony tissue from
the alveolar ridge
b-buccal fold

In the region of the median line tumour tissue is present

In the original tumour region no vital tumour tissue is evident, only atrophic mucoperiostium and keratin pearls

factor of 2) tissue absorption than did intravenous administration. For the skin, there was an even higher tissue absorption (factor of 3) with intra-arterial administration.

Table I (from Didolkar, 1978) Tissue concentrations of Adriamycin (microgr/kg) in biopsies taken 1 hour after administration of Adriamycin (2-4mg/kg).

Organ	isolated perfusion	intra-art. infusion Ipsi-lat.	contralat.	i.v. infusion
muscle	15	0.6	0.4	0.3
fat	10	0.3	0.2	0.3
nerve	6	0.4	0.8	0.8
skin	5	0.75	0.25	0.25
blood	0.02	12.1	0.43	1.1
bonemarrow	2.6	-	-	-

In this animal study, however, the femoral artery was chosen for the intra-arterial infusion, and this vessel takes too great a fraction of the heart minute volume to allow maximal utilization of the superior effectiveness of intra-arterial infusion. In any case the flow in the target area artery (α) must be as low as possible.

Anderson et al. (1970) carried out a comparative study with intravenous and intra-arterial administration in patients, and could demonstrate a greater concentration of Methotrexate in leiomyosarcoma tissue after intra-arterial administration than after intravenous administration of equal dosages.

Bier et al. (1983) determined the tissue distribution in four groups of sheep infused with 57 Co-Bleomycin by various routes:
1. right transverse facial artery with the catheter occluding the vessel (flow≈0)
2. local seletive intra-arterial infusion via the right superficial temporal artery
3. intra-arterial infusion of all the branches of the right external carotid artery
4. right saphenous vein (intravenous administration).

The dosage was 0.3 mg/kg body weight of Bleomycin. After 6 hours, the tissue concentration of the 57 Co-Bleomycin in the right buccal area was measured. The following values (in microgr/gr tissue) were found:

Table II

	Catheter position			
	1.	2.	3.	4.
right buccal region	0.374	0.141	0.148	0.146
left buccal region	0.157	0.122	0.127	0.140

Table II shows that after 6 hours the benefit of intra-arterial administration compared to intravenous administration has almost completely disappeared (from 0.14 to 0.12 microgr/gr tissue of the buccal region). Only in group 1 is there a difference between right (i.a. bolus) and left (systemic) levels, which differ by a factor of 2.3. The vessel occluding catheter position (group 1) is however not relevant for head-neck tumours, where this catheter position results after a short time in thrombosis and necrosis of the artery. The objections to this experimental study method of comparing intra-arterial administration with systemic administration include: a bolus injection was used, the determinations were made after 6 hours so that the superior effectiveness of intra-arterial versus intravenous administration was almost eliminated, and tissue absorption in a hypothetical tumour location is not the same as in tumour tissue (Fujimoto, 1974; Lee et al., 1980). This study does demonstrate that the flow in the target area artery is one of the most important factors in determining the superior effectiveness of intra-arterial infusion over intravenous administration.

Lee et al. (1980) carried out an investigation into the distribution of labelled Adriamycin in the tissues of patients. The absorption of the Adriamycin was greatest in the tumour and the liver, and thereafter in decreasing order, lymph vessels, muscle and bone marrow, and the lowest concentrations were measured in fatty tissue and skin. According to Lee et al. (1980), plasma concentrations are not a good indicator of biological effect at the cellular molecular level. Higher concentrations of Adriamycin metabolites were detected after intra-arterial administration than after intravenous administration. He explained this by a higher biological activity in the target area.

One of the most important comparative studies on the intra-arterial versus intravenous administration was carried out by Schouwenburg et al. (1980). By tracing the effect on a known transplanted tumour in a host when only the administration mode is varied (local selective intra-arterial versus systemic), he proved superiority of the intra-arterial administration mode. In the animal model of Schouwenburg, the intra-arterial administration is analogous to that of the human model of Bilder (1968). Three cytostatic agents were tested as mono- and multi-drug therapy and in different

concentrations and sequences. The effect on the tumour was measured by percentage volume reduction. In this study Bleomycin has a greater (factor 3.75) effect when intra-arterially administered than when intravenously administered up to the "plateau concentration" for the tumour in question.

A plateau concentration for 5Fu does not appear to exist; for all administered concentrations, a higher tumour volume reduction (by a factor of 3) was measured with intra-arterial than with systemic administration. Methotrexate had no effect on the tumour used and no advantage factor could thus be determined.

With simultaneous administration of the combination 5Fu, Methotrexate and Bleomycin using a continuous intra- arterial infusion over 7 days, no greater tumour reduction on the regionally treated side was evident than when Bleomycin was administered as monotherapy by regional intra-arterial infusion. There was thus indication for neither additive effects nor potentiation of the drugs when infused as a simultaneous "cocktail".

At a dosage at which toxicity was reduced to an acceptable level, intermittent continuous intra-arterial local selective infusion using polychemotherapy achieved an extremely important anti-tumour effect which was superior to monochemotherapy; the tumour growth inhibition also lasted longer. Multidrug regimes in this study gave better results only when an intermittent sequence was used. The intra-arterial infusion route resulted in a higher response rate than that obtained in the systemically treated contralateral tumour area.

4. Summary.

Pharmacokinetic studies supported by clinical observations have demonstrated that intermittent continuous local selective intra-arterial infusion with cytostatic agents offers a therapeutic advantage over systemic administration. In order to utilize the superior effectiveness of intra-arterial infusion ($\overset{\varphi}{R}d$), intra-arterial bolus administration must not be used. There are however further reasons why a difference between clinical results and the theoretical superior effectiveness of intra-arterial infusion ($\overset{\varphi}{R}d$) may be observed:

a. There is considerable individual tumour-dependent variability in the effectiveness of the cytostatic agents used. Some dose-survival curves rise less steeply in the lower concentration range (large D_0), so that the expected effect is reduced.
b. The calculated superior effectiveness of intra-arterial

infusion ($\bar{R}d$) (in a homogeneously vascularized tumour with constant microvascular architecture throughout) should be evidenced in the whole tumour. For most solid tumors, however, this is not the case (Schmidseder and Noma ,1977; Tannock, 1968; Pavelic et al., 1981; C.I.B.A., 1983).

The animal study of Schouwenburg et al. (1980) shows conclusively that intermittent continuous local selective infusion (provided that the chemotherapeutic agent affects the tumour) results in a clearly greater effect on the tumor than does the systemically administered chemotherapeutic agent.
It can be histologically shown that concurrent combination treatment of radiotherapy and chemotherapy, in a given tumour, results in complete disappearance of tumour tissue on the intra-arterially infused side of the tumour, in contrast to that part of the tumour which is chemotherapeutically treated by recirculation of the agent.

PART II

ORIGINAL INVESTIGATION

CHAPTER X

MATERIALS AND METHODS

Case Histories of Patients Treated

Acknowledgement

These studies were made possible only with the close cooperation and combined team approach of the Departments of Maxillo-facial Surgery, Radiotherapy, Radiobiology, Pathology and staff of many other disciplines at The State University, Utrecht.

1. Staging and Selection of Patients.

The described method was only used for patients for whom there was no chance or extremely limited chance of cure through combination surgery and/or radiotherapy. The extension of the tumour had already reached or penetrated vital structures which made radical resection impossible. Through improved surgical reconstructive possibilities in recent years, the indication, "non-resectable" has acquired a different meaning and is applied relatively less often (Pearlman et al., 1982). It is for this reason that only patients with a very poor prognosis were included in this investigation. A comparison with respect to survival after treatment with other therapies, which usually involved AJC 1978 and UICC 1978 Stages III and VI, is hardly, if at all, possible. This study thus concerns a final treatment, the ultimate aim of which was cure in a patient group which would otherwise be considered for palliative treatment only.

Selection of patients:

A. Non-resectable oral cavity, oropharynx and maxillary squamous cell carcinomas of all histological grades (Broders, 1920) of all N stages, without demonstrable distant metastasis.

Non-resectability was defined as follows:
1...Squamous cell carcinomas of the maxilla with expansion to, or growth into:
- a. Skull base via the retromaxillary region (growth into and through the lamina pterygoidea and maxillary tuberosity).
- b. The uppermost ethmoid region and/or infiltration into the most rostral skull groove (the orbitomaxillo-ethmoidal angle, the olfactory region, the sphenoidal sinus and/or frontal sinus).
- c. The parapharyngeal area, to or into the prevertebral fascia and/or penetration into the neurovascular bundle.

2. Squamous cell carcinomas of the tongue and floor of the mouth with extension to or into:
- a. The hyoid, with penetration over the mid-line in the tongue base-hyoid region.
- b. Parapharyngeal area, to or into the prevertebral

fascia and/or penetration into the neurovascular bundle.

3. Squamous cell carcinomas of the oropharynx/retromolar trigonium/cheek with extension to or infiltration into: the parapharyngeal area, to or into the prevertebral fascia, and/or infiltration into the neurovascular bundle.

Determination of non-resectability:

After histopathologic diagnosis of the tumour biopsy, the feasibility of radical resection is determined by:

a. clinical evaluation;
b. staging of the tumour under general anaesthesia when uncertainty exists concerning tumour extension;
c. computer tomography.

Exclusion of distant metastasis:

a. Chest radiographs and when necessary, computer tomography.
b. Bilirubin, transaminases (SGOT/SGPT), and alkaline phosphatase. In patients with increased levels (bilirubin > 17 umol/l, SGOT > 30 U/l, SGPT > 30 U/l, alkaline phosphatase > 140 U/l) bone and liver scans were conducted.

B. Patients were excluded from concurrent combination treatment if:

1. active uncontrolled infections were detected;
2. other serious non-malignant systemic diseases existed which would place the patient at risk for the treatment;
3. malignancies other than a second oral cavity carcinoma of the same histological grade were present;
4. overt psychosis or marked senility was present;
5. prior radiotherapy or any form of chemotherapy had been given;
6. leucocyte count of less than 4 Gi/l and platelet count less than 100 Gi/l;
7. there was compromise of renal function, as defined by serum creatinine of more than 133 umol/l;
8. bilirubin greater than 17 umol/l;
9. there was compromised lung function as determined using simple spirometry.

C. An age limit was not initially set, although on the basis of the present policy, patients older than 75 years should be excluded. The Karnofsky index of the patients was arbitrarily set at greater than 50 (Appendix IV).

D. Informed consent and cooperation for the planned therapy was obligatory.

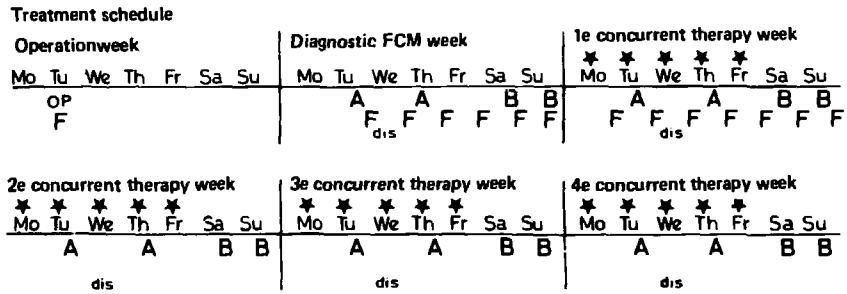
2. Materials and Methods.

2.1. Cytostatic Agents.

The cytostatic agents Adriamycin and Bleomycin were administered following the local selective intra-arterial "intermittent-continuous" method (Part I, Chapter IX). The choice of the cytostatic agents Adriamycin and Bleomycin for the concurrent combination treatment with radiotherapy was made on the grounds of the literature study on combination treatments (Part I, Chapter VIII) and the experimental studies of these combination treatments (Part I, Chapter IV, 3.2. and 3.3.).

The actual combination treatment began in the first concurrent therapy week (see treatment regime). Administration of the cytostatic agents by infusion took place betwen 8.00 p.m. and 8.00 a.m the following morning (12 hours continuously), so that the patient's freedom of movement was minimally disturbed.

2.2. Treatment Regime.



F = biopsy for flow cytometry
A = Adriamycin (Doxorubicin) 15 mg over 12 hours by intra-arterial continuous infusion.
B = Bleomycin 15 mg over 12 hours by intra-arterial continuous infusion.
✱ = radiotherapy 200 cGy
1 hour after termination of i.a. infusion
OP = operation day
a. cervical lymph node dissection
b. catheterization and selective ligation
dis = disulphine blue control of the infused area.

In order to prevent thromboembolic processes occurring, 500 mg Ascal (Acetylsalicyl-acid) and low dose Heparine 10.000 U/day is administered. Hyperalimentation, generally applied by way of a mini gastric tube, is required after the second concurrent therapy week.

2.3. Intra-arterial Cannulation Technique.

2.3.1. Introduction.

Cannulation of the external carotid artery by a direct operative approach can be achieved by:
1. Direct cannulation of the common carotid (Sullivan et al., 1953, 1961, 1962, 1965).
2. Orthograde cannulation via branches of the external carotid (Sullivan et al., 1959, 1961; Westbury et al., 1962; Espiner, 1962 and Espiner and Westbury, 1964).
3. Retrograde cannulation via the superficial temporal artery (Westbury et al., 1962; Espiner, 1962 and Espiner and Westbury, 1964.
4. Retrograde or orthograde cannulation of the external carotid artery with ligation of the vessels not supplying the tumour region (Bilder, 1968; Kreidler, 1976) = local selective infusion.

Direct catheterization via the common carotid artery with advancement into the external carotid in order to achieve local selective infusion of the tumour region was never necessary in our study. Preference is for retrograde cannulation of the superficial temporal artery. If this was shown to be impossible due to sclerosis of the superficial temporal artery (usually accompanied by a very convoluted course) or when obliteration of the artery had occurred due to thrombosis or penetration by the primary tumour, then retrograde cannulation of the facial or occipital artery was opted for.

2.3.2. Cannulation Technique.

A. Technique for Direct Cannulation of the Common Carotid Artery.

In our study, it was never necessary to use this technique; there was always a branch of the external carotid artery which could be cannulated.

For the sake of completeness, the technique for direct cannulation of the common carotid artery which is applied when other cannulation possibilities are excluded, is described.

Sullivan's (1962) cannulation technique is by means of a polyethylene catheter attached to a curved needle. The

catheter is introduced into the vessel and then brought out again using the curved needle (the direction of insertion is the same as that of blood flow). After the catheter has been pulled through, the catheter needle is cut off. The catheter is pulled back so that the tip slips into the vessel. The fixation of the catheter to the common carotid artery (or the external carotid) is achieved by means of tunnel fixation and purse string suture at the point of insertion.

When the Braun Melsungen catheter is used, the above described method is not applicable. Then it is necessary to apply u-shaped artery forceps to the common carotid and external arteries so that the circulation of the internal carotid is not completely shut off. After the placement of a purse string suture and opening of the vessel wall, the catheter is introduced and advanced to the desired position after which the purse string is tied. The catheter is then fixed to the carotid artery wall using tunnel fixation after which the artery forceps can be removed.

B. Orthograde cannulation technique (2.3.3., Schemas VIII and IX).

After freeing of the superior thyroid artery (in oral cavity tumours this branch of the external carotid artery does not contribute to the blood supply of the tumour) the artery is elevated with "arterial loops". Scandicaine 3% is injected to avoid spasm. After elevation of the tied distal loop, a tangential opening is made in the wall with sharp curved scissors; the intima is then hooked with a small sharp hook so that the catheter can not be manipulated between the intima and adventitia. The catheter (Braun Melsungen) filled with heparinized physiological saline is then advanced. If the superior thyroid artery curves too abruptly in a cranial direction after branching from the external carotid artery, sufficient mobilization (in a caudal direction) must be achieved so that the tip of the catheter passes easily (cranially) into the external carotid artery. The catheter is now advanced until its opening is directly under the vessel which supplies blood to the tumour. The other branches of the external carotid artery have been elevated cranially (Bilder, 1968). After checking of the perfusion area with disulphine blue, the elevated vessels are ligated.

C. Retrograde cannulation technique.

The parietal branch of the superficial temporal artery is freed through a pre-auricular incision (Fig. 1.) (Espiner, 1962) (2.3.3. Schemas I, II, III). The artery usually lies in front of or under the accompanying vein. Scandicaine 3% is infiltrated in order to prevent spasm (Fig. 1.). The artery must be freed as far caudally as possible because the greatest curvature of the vessel is found in the region of


1


2


3


4


5


6

the zygomatic arch. Elevation of the vessel is now very simple and the chance of perforation by the catheter is minimized.

After elevation of the vessel, the artery is opened tangentially with sharp curved scissors, the intima is then hooked and the Braun Melsungen catheter is introduced (Fig. 2.). Several anatomical variations can cause problems in advancement of the catheter, and the sharp curve in the area of the zygomatic arch has already been mentioned. The second difficulty usually occurs at about 5 cm. The so-called "Rideau Stylien" occurs at this point (Snow, 1966), where the artery assumes an S-form because it is bordered by the stylomandibular ligament medially, and by the posterior belly of the digastricus muscle laterally. If the catheterization is hampered at this point, manipulation of the head usually results in sufficient extension of the vessel to allow further advancement of the catheter (using a delicate rotatory movement) to a point just caudal to the vessel perfusing the tumour. After freeing the branches of the external carotid artery, the vessels which do not perfuse the tumour are looped (Figs 3 and 4). After palpation of the tip (this is possible in carcinomas in which the lingual or facial artery must be perfused, and impossible in perfusion of the maxillary artery) disulphine blue injection is carried out. If the tumour region becomes completely blue (Fig. 5.), the vessels which do not perfuse the tumour can be ligated. Similarly, the external carotid artery is ligated above the lateral opening of the catheter tip if the tumour location allows this (2.3.3., Schema V.). Fixation of the catheter relies mainly on "biting sutures" in the skin at the temporal

---

Fig. 1

1. The parietal branch of the superficial temporal artery is freed via a pre-auricular incision.

2. Scandicaine (3%) is infiltrated to prevent vessel spasm.

Fig. 2
After elevation of the vessel, a sharp curved scissor is used to open the vessel tangentially, after which the intima is hooked and the Braun Melsungen catheter introduced.

Fig. 3
After freeing the branches of the external carotid artery, the branches which do not perfuse the tumour are elevated.

Fig. 4
Detail of Fig. 3.

Fig. 5
The bright blue colour is visible in the target area directly after intra-arterial infusion of disulphine blue.

Fig. 6
McFee incision in radical neck dissection. Disulphine blue staining is visible submandibularly.

exit site. The ligatures around the cannulated vessel, display some loosening during the course of treatment, probably from necrosis of the artery at ligation sites and the effect of chemotherapy on the arterial wall in this area.

In retrograde cannulation, the position of the head can influence the site of the catheter tip in the external carotid. With flexion of the head to the catheterized side, the tip penetrates further, and by movement of the head away from this side, the tip comes to lie higher in the external carotid. In cases of ligation cranial to the tip, this phenomenon will be unable to occur, at least in the first weeks.

Advantages of retrograde versus orthograde cannulation are:
1. In retrograde cannulation, the chance of after-bleeding when the catheter is removed at 5 weeks, is less.
2. If, in a negative neck (N_0) catheterization is necessary because of the non-resectability of the primary tumour, surgery of the neck as primary treatment can be avoided.

Another possibility for retrograde cannulation in oral cavity carcinomas, is via the occipital artery or the facial artery when the extension of the primary tumour allows this (2.3.3. Schemas V, VI, VII).

2.3.3. Schematic Representation of Local Selective Intra-arterial Infusion (Bilder, 1968).

Schema I.

Retrograde selective cannulation via the superficial temporal artery of the lingual artery, allowing perfusion of:
1. lingual artery deep lingual artery
2. ascending pharyngeal artery

▲ ligation to allow selective infusion (Bilder's ligature).

⍋ entry site of the catheter in the cannulated vessel = ligature/fixation to the vessel.

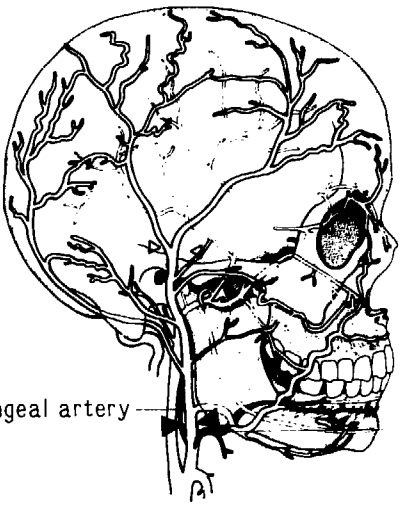
△ fixation of the catheter to the skin by sutures at the exit site.

Schema II.

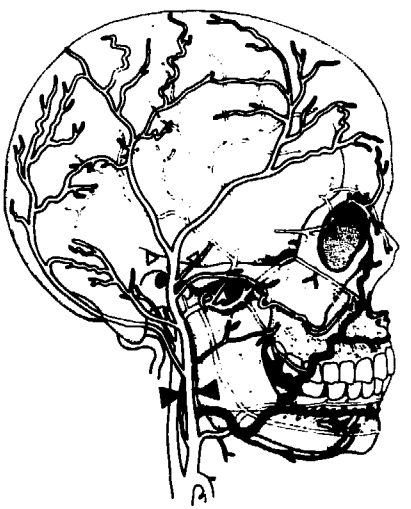
Retrograde selective cannulation via the superficial temporal artery of the facial artery, allowing perfusion of

facial artery
- submental artery
- mandibular labial artery
- maxillary labial artery
- angular artery

Schema I



Schema II



Schema III.

Retrograde selective cannulation via the superficial temporal artery of the maxillary artery.

1. retroauricular artery

2. maxillary artery
   - deep auricular artery
   - mandibular alveolar artery
   - masseteric artery
   - medial meningeal artery
   - deep temporal artery
   - buccal artery
   - caudal maxillary artery
   - descending palatine artery
   - major palatine artery
   - incisive artery
   - infra-orbital artery
   - nasofrontal artery

3. zygomatico-orbital artery

4. medial temporal artery.

Schema IV.

Retrograde selective cannulation via the superficial temporal artery of the

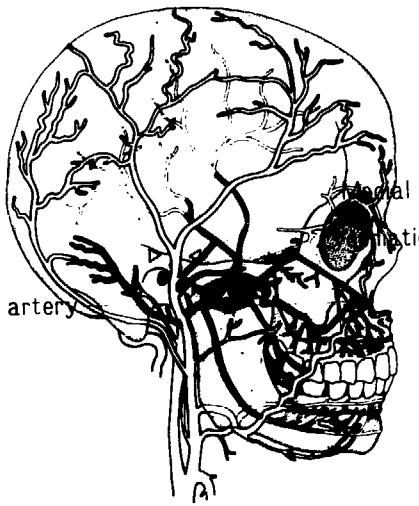
1. lingual artery

2. facial artery

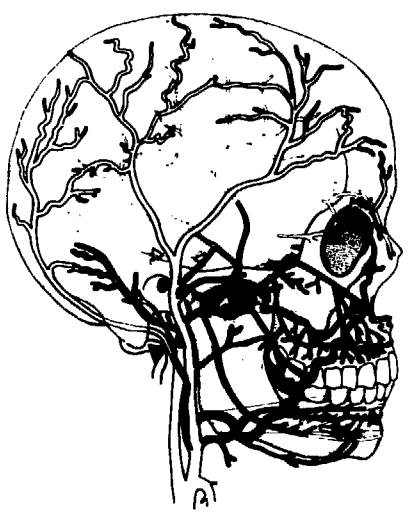
3. maxillary artery

allowing combined perfusion of the areas of Schema I, II and III.

Schema III



Schema IV



Schema V.

Retrograde selective cannulation via the occipital artery of the lingual artery, allowing perfusion of the

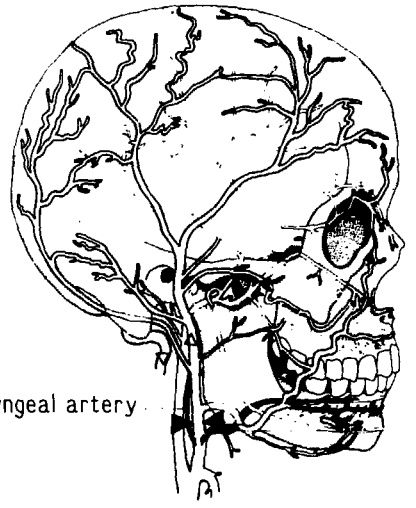
1. lingual artery — deep lingual artery
2. ascending pharyngeal artery

Schema VI.

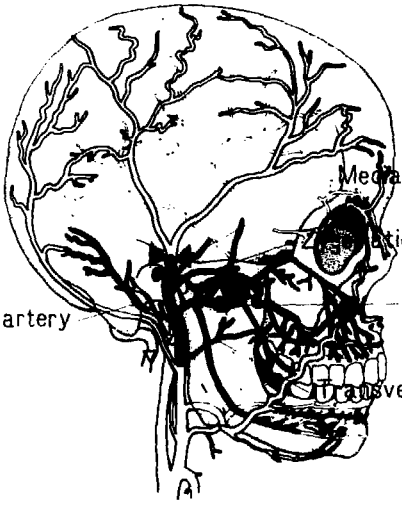
Retrograde (selective) cannulation via the occipital artery of the maxillary artery, allowing perfusion of

1. transverse facial artery
2. retroauricular artery
3. maxillary artery
   - deep auricular artery
   - mandibular alveolar artery
   - masseteric artery
   - medial meningeal artery
   - deep temporal artery
   - buccal artery
   - caudal maxillary artery
   - descending palatine artery
   - major palatine artery
   - incisive artery
   - infra-orbital artery
   - nasofrontal artery
4. zygomatico-orbital artery
5. medial temporal artery.

Schema V



Schema VI



Schema VII.

Retrograde selective cannulation via the facial artery of the lingual artery, allowing perfusion of

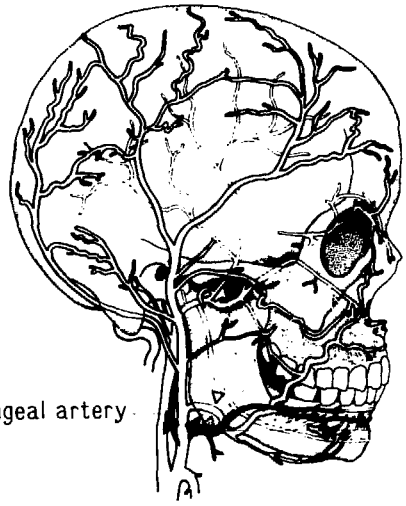
1. lingual artery — deep lingual artery
2. ascending pharyngeal artery.

Schema VIII.

Orthograde selective cannulation via the cranial thyriod of the lingual artery, allowing perfusion of

1. lingual artery — deep lingual artery
2. ascending pharyngeal artery

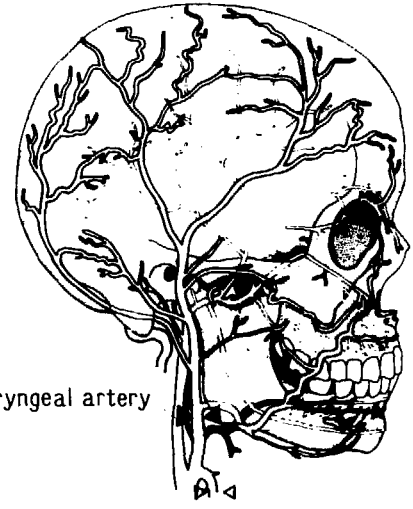
Schema VII



Ascending pharyngeal artery

Lingual artery

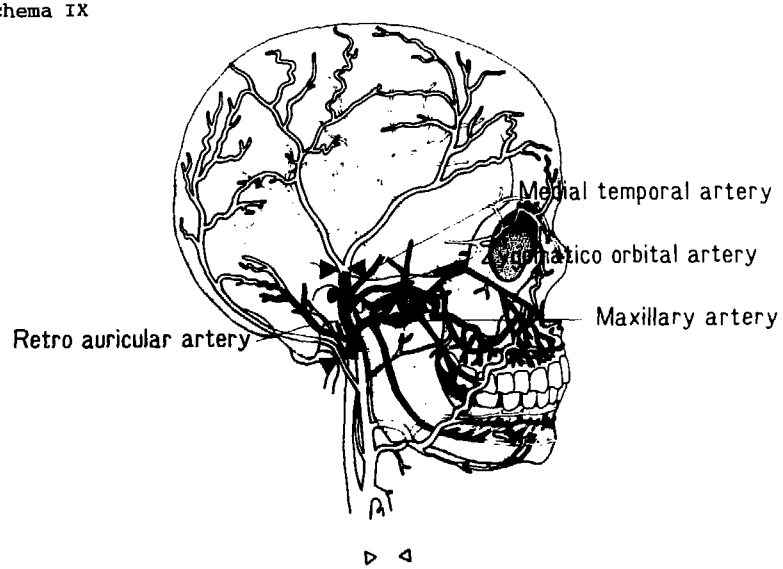
Schema VIII



Ascending pharyngeal artery

Lingual artery

Schema IX



Schema IX.

Orthograde selective cannulation via the cranial thyriod artery of the maxillary artery, allowing perfusion of

1. (inconstantly) transverse facial artery
2. retroauricular artery
3. maxillary artery
   - deep auricular artery
   - mandibular alveolar artery
   - masseteric artery
   - medial meningeal artery
   - deep temporal artery
   - buccal artery
   - caudal maxillary artery
   - descending palatine artery
   - major palatine artery
   - incisive artery
   - infra-orbital artery
   - nasofrontal artery
4. zygomatico-orbital artery
5. medial temporal artery.

2.4. The Infusion System: Description and Nursing Aspects.

Catheter description.

The Braun Melsungen Catheter, 1.5 x 2.1 mm diameter, is made of polyethylene, and is calibrated at 5, 10, 15 and 20 cm to facilitate its passage to the correct catheter tip position in the external carotid artery. The catheter tip is closed with a round end, and the opening is on the side of the catheter, 5 mm from the tip. In the periods between intra-arterial infusion with cytostatic agents, the catheter is always connected to a flush line which allows a flow of 3 ml/hour (Sorenson intraflow continuous flush Cat CFS 03F). The flush line is connected to a pressure (300 mm Hg) reservoir which contains 500 ml NaCl 0.9% to which 5000 units of Heparin is added. In addition to the continuous flush (3 ml/hour), a rapid flush (5 drops every 2 hours) is manually administered by nursing staff via the Sorenson continuous flush system. This is more for checking the whole intra-arterial system for nursing purposes, than for prevention of thrombosis. The following parameters are checked:

1. pressure of the pressure reservoir (greater than 300 mm Hg)
2. no air in the system
3. no leakage at connection areas, taps and joints
4. sufficient NaCl/Heparin in the pressure reservoir
5. no blood in the arterial line
6. good attachment of the arterial lines to the skin by plaster

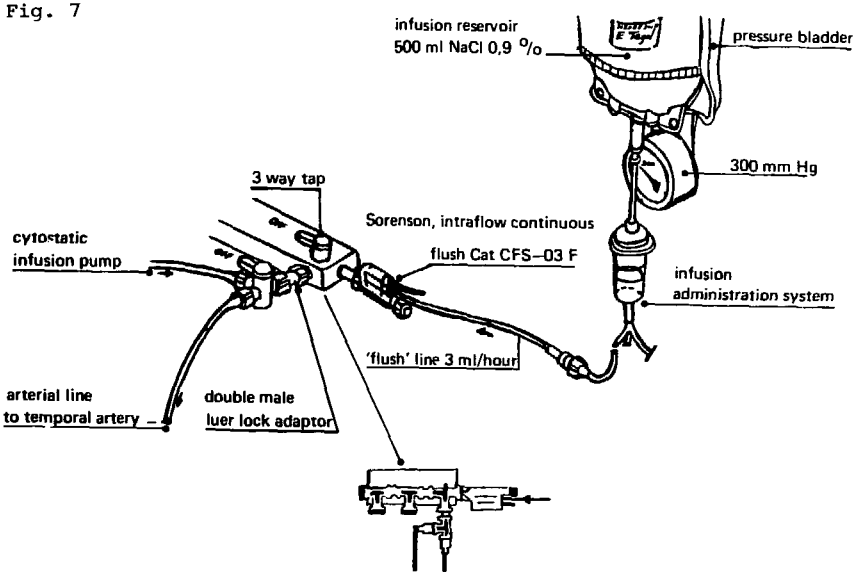
Figure 7 is a schematic representation of the infusion system used in the majority of patients. For the last patients in the series, the intra-arterial catheter was attached to a Cormed minipump, with a maximal flow of 50ml/24 hours.

The arterial lines were changed every 48 hours by the doctor on duty. The cytostatic agents were administered by a K.10 infusion pump (Hospital Medical Corporation, Colorado, U.S.A.). 15 mg Adriamycin or Blemoycin (or 7.5 mg for a double arterial line) was dissolved in 60 ml 0.9% NaCl. The pump was regulated so that the 60 ml was infused over 12 hours. The nursing staff was responsible for the whole infusion period for protocols involving checking of:

1. patient
2. infusion system
3. pump
4. disconnection of the cytostatic agent pump after the cytostatic infusion was complete.

Control of the perfusion area using disulphine blue took place once per week or more often when indicated (e.g. pain during rapid flushing, pain during administration of the cytostatic agent).

Fig. 7



Schematic representation of the infusion system used in the majority of patients.

2.5. Radiotherapy.

Radiotherapy was always carried out some hours (1 - 2 hours) after the termination of intra-arterial infusion. In order to achieve the optimal sequence of chemotherapy and radiotherapy, the interval should be short (Part I, Chapter IV, 3.2. and 3.3.).

Radiotherapy was carried out with a linear accelerator (8 MeV), 200 cGy per fraction, five fractions per week up to a total dose of 4000 cGy. If local toxicity after 4000 cGy was grade III or less, 5000 cGy was administered.

2.6. Cervical Lymph Node Dissection.

Because cervical lymph node metastases subhyoidally fall outside the selective perfusion area, it is inadmissible to omit cervical lymph node dissection in cases of palpable cervical metastases lower in the neck. In the present study treatment was thus always begun with cervical node dissection in cases of clinically positive cervical lymph nodes. The cranial branch of the accessory nerve was spared if possible, depending on the location of the palpable lymph nodes in the superior neck region, and the cervical branches of the accessory nerve could usually be saved.

Exhaustive dissection of the submandibular area in cases of infiltration of the primary tumour in this region (when tumour and submandibular metastasis are continuous) is not necessary because the regional selective perfusion is maintained suprahyoidally if the most ventral branches of the external carotid artery are perfused.

In order to guarantee an effective local chemotherapy, in the disulphine blue testing, the area cranial to the digastricus muscle must stain blue (Fig. 6.). Access to the neck in concurrent combination treatment is always achieved following McFee's approach in order to avoid a submandibular T-shaped incision.

2.7. Flow Cytometry.

The purpose of flow cytometric determinations has already been outlined in the summary of Part I, Chs II, III and IV. To prove the possible correlation between the treatment-induced cell cycle perturbation and the chance of sterilization of the local regional tumour process, daily tumour biopsies were taken in the first week of, and the week prior to, the concurrent combined treatment.

2.7.1.Biopsy Procedure.

In order to evaluate samples which are regarded to be representative of the tumour, biopsies were taken from the periphery of the tumour (the site of the largest growth fraction of the tumour), and at the same time care was taken that the biopsies were from the same quadrant of the tumour. All the tumours were so large that there was ample material available during the first weeks of the combination treatment. Also, there was in general little pain involved in this sampling.

The incisional biopsies (or biopsies taken with forceps) were immediately placed in phosphate buffered saline (PBS) and stored at 4 degrees C.

2.7.2.Mechanical and Enzymatic Preparation of the Cell Suspension.

Cell suspensions were prepared according to the method of Rutgers (1981).

2.7.3.DNA Staining.

After RNase treatment, DNA is stained by adding 0.15 ml ethidium bromide (EB) solution (1 mg EB/ml) to the cell

suspension. The cell suspension is then passed through a filter with pores of 70 micrometers. The cell suspension is then ready for flow cytometry. The flow cytometer used was a cytofluorograph 4802A (Bio/Physics Inc.).

2.7.4.Histogram Analysis and DNA Index Determination.

Eliminating the so-called "background" from the DNA histograms is done by drawing a line through a point left of the base of the G_1 peak to the right base of the G_2+M peak, on a log-log representation of the histogram. In this way, unwanted counts under this line could be separated from the histogram. This was done with the aid of an interactive computer programme (van der Linden, 1981).

2.8. Treatment Evaluation after Administration of Half the Computed Dose.

A. Evaluation of the treatment took place after administration of half of the computed dose (TD 2000 cGy) (2 weeks combination therapy, Matsamura and Motomura, 1973). A sufficient clinical response must by then have occurred in order to justify further treatment (biological selection, see also Part I, Chapter VIII). Clinical response was scored as partial or complete remission. Complete remission was defined as a complete disappearance of clinical indications of the tumour (no induration, ulceration, fistula or skin defects, although return of normal cortical bone structure in follow-up radiographs was not a requirement). Partial remission (50% or more) was defined as a decrease in tumour area greater than or equal to 50% (multiplication of the longest diameter by its perpendicular diameter).

B. Obvious changes in the distribution of tumour cells over the different cell cycle phases and an accumulation G_2+M phase caused by the combination treatment, may be related to local tumour sterilization (Part I, Chs III and IV).

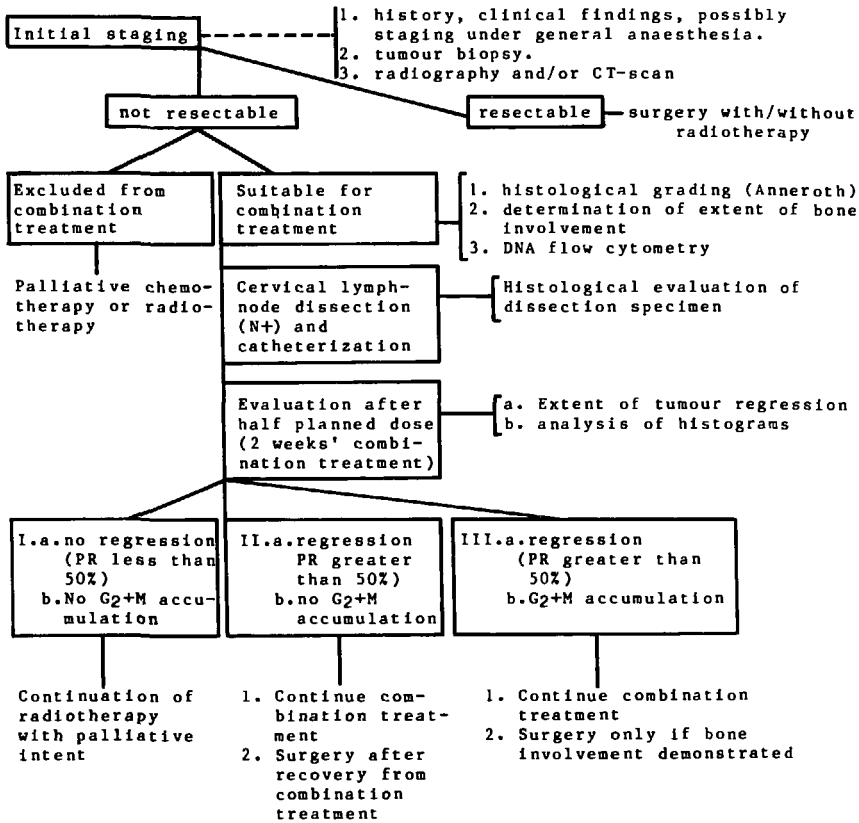
C. Modification of the cytostatic agent on the basis of toxicity. If the leucocyte count was less than 3 Gi/l or the platelet count was less than 75 Gi/l, Adriamycin treatment was postponed for one week or longer until haematological recovery to these levels occurred (< Grade 2, Appendix III). The combination therapy was then continued with Bleomycin.

Based on information gained from the first patients treated, subsequent patients were treated with by a combination of I, II and III of the Decision Chart (Fig. 8).

I. If a partial response of less than 50% was recorded, and no, or little evidence of cell cycle perturbations was seen, further combination treatment was abandoned because cure seemed unlikely (the tumour will probably remain non-

Figure 8. Decision Chart

Decisions during the concurrent combination therapy in non-resectable oral cavity carcinomas.



resectable). Further concurrent combination treatment could not be justified when weighed against the increased mortality of the treatment (palliative chemotherapy or radiotherapy could still be considered).

II. If, after 2 weeks' concurrent combination treatment, there was clinically demonstrable response of 50% or more, and no, or little evidence of cell cycle perturbations was seen, subsequent surgery directly following the first half of the combination treatment, or further combination treatment with subsequent surgery after recovery from the mucositis and dermatitis, were considered.

III. If there was clinical evidence of response and distinct cell cycle perturbations, the concurrent combination treatment was continued because cure may be possible.

2.9. Histological Grading of Tumour Biopsies at Initial Staging.

From the *retrospective Dösak study* (Platz et al., 1982, 1983, 1985) univariate analysis showed a relationship between prognostic significance (survival) and the extent of histological differentiation (obvious keratinization as opposed to no keratinization, and anaplasia). In multivariate analysis, however, no relationship was found (Part I, Ch. I, 5.3.).

Classification according to Broders (1920) was of limited value for prognosis and therapy in the studies of Arthur and Fenner (1966), Arthur and Farr (1972,) and Bethmann and Heinrich (1965). Jakobsson (1973) developed a multifactorial histological grading system, and in a study of larynx carcinomas, found a correlation between prognosis and evaluated histological parameters. Using Jakobsson's (1973) method, Crissman et al. (1980) studied carcinomas of the floor of the mouth and found a correlation between the number of local regional recurrences and an increased stage of invasion (Jakobsson, 1973).

Anneroth and Hansen (1984) modified Jakobsson's method and their evaluation system is as follows:

A. Grading of tumour malignancy by histological classification of the tumour cell population.
I. Structure (the extent of the tumour cells' cohesiveness
(4 grades).
Grade 1 - in solid sheets and/or in papillary configuration
Grade 2 - long narrow strands and bands
Grade 3 - tumour invasion in the form of minute groups of cells due to excessive loss of

cellular cohesiveness
Grade 4 - marked dissociation of tumour cells

II. Keratinization tendency (4 grades)
Grade 1 - large amounts of keratin
Grade 2 - some keratinization apparent
Grade 3 - minimal keratinization
Grade 4 - no keratinization

III. Nuclear aberrations (4 grades) involve variations in size and shape of tumour cell nuclei, increased nuclear/cytoplasm ratio, presence of hyperchromatic and multiple nuclei, and atypical mitosis.
Grade 1 - few nuclear aberrations in a relatively homogeneous cell population
Grade 2 - moderately abundant nuclear aberrations
Grade 3 - abundant nuclear aberrations with few large anaplastic nuclei
Grade 4 - abundant nuclear aberrations and numerous large immature anaplastic nuclei rich in chromatin.

IV. The number of mitotic figures observed in one high power (450x) microscopic field (4 grades)
Grade 1 - few (0 - 2) but more than normal
Grade 2 - moderate numbers (3 - 4) of mitoses
Grade 3 - numerous numbers (5 - 6)
Grade 4 - extremely numerous (more than 6)

B. Grading of tumour malignancy by histological classification of the tumour-host relationship.

I. Mode of invasion (4 grades)
Grade 1 - well-defined basement membrane
Grade 2 - less distinct basement membrane
Grade 3 - no distinct basement membrane visible
Grade 4 - no distinct basement membrane visible, presence of diffuse infiltration

II. Stage of invasion (4 grades)
Grade 1 - microinvasion
Grade 2 - invasion of the lamina propria
Grade 3 - invasion through the lamina propria into muscle and gland tissue
Grade 4 - massive invasion with replacement of stromal tissue with tumour cells

III. Inflammatory response. The occurrence of infiltration of plasma cells and lymphocytes in close relation to invasive tumour cells was subjectively evaluated.
Grade 1 - marked
Grade 2 - moderate
Grade 3 - slight
Grade 4 - no inflammatory infiltration

On the basis of Anneroth's (1984) classification (above) the primary biopsies of the patients in the present study were retrospectively evaluated by an oral pathologist. In our

patient material the tumours were always in Grade 4 invasion stage (B. II).

2.10. Histological Evidence of Lymph Node Metastasis.

All patients with clinically suspected cervical lymph node metastases underwent a radical cervical node dissection on the homolateral side of the primary tumour and where necessary a supra-(omo)-hyoidal dissection on the contralateral side (with preservation of the internal jugular vein).

In cases of clinically negative neck, a surgical staging was always carried out during the insertion of the catheters. In ligating the external carotid artery branches which do not supply the tumour region, it is simple to gain access to the neurovascular bundle to facilitate taking frozen sections from the nodes adjacent to the internal jugular vein.

The neck dissection specimen was prepared as follows. Inspection and palpation of the specimen is the first stage, and all lymph nodes were dissected out. The jugular vein was then opened and the clot removed, after which the vessel wall was inspected and palpated. If lymph nodes were encountered then the possibility of penetration and/or adhesion of the lymph node with the vessel wall was investigated by dissection of the node. The whole preparation was then sliced into thick sections, and all lymph nodes thus exposed were macroscopically examined together with their relationship with surrounding anatomical structures. The lymph nodes were then dissected so that the capsule of the nodes remained as far as possible intact. Depending on their size, the nodes were sectioned at several levels so that they could be studied for the presence of tumour tissue. The anatomical location of the nodes (submental, submandibular, retromandibular, high, mid and low cervical, and supraclavicular) was also recorded. After histological processing, the lymph nodes were then examined microscopically for the presence of tumour, capsular invasion and capsular penetration.

2.11. Radiological Determination of Evident Tumour Invasion of Bone.

Radiographs of all the patients were made in order to give as clear as possible a picture of the extent of spread of the tumour in hard and soft structures. For the maxilla, dental radiographs and occlusal films, and where necessary, radiographs of the paranasal sinuses, planigrams and CT-scans were made. For the mandible, dental radiographs, occlusal radiographs, OPG's and where necessary CT-scans were made. Bone invasion was classified into three categories:

1. No demonstrable bone invasion.
2. Bone invasion by the tumour manifested by irregular cortical borders of the bony structures.
3. Bone invasion by the tumour which was manifested as a continuity defect of the mandibular cortex, or massive destruction of the maxillary tuberosity, zygomatico-alveolar process, zygomatico-frontal process and/or a continuity defect of the alveolar process and hard palate extending to the paranasal sinuses and/or destruction of bones of the skull.

3. Case Histories of Patients Treated.

The following is a chronological description of patients admitted to the Department of Maxillo-Facial Surgery at the State University of Utrecht and treated by concurrent combined chemotherapy and radiotherapy, followed in some cases by salvage surgery.

Case History 1.

Male, 76 years.

The patient was admitted in May 1982 with an ameloblastic carcinoma of the mandible. The tumour had extended through the skin surface, the mandible showed a spontaneous fracture, and there was extensive infiltration from the floor of the mouth and base of the tongue over the mid-line. In the base of the tongue, the tumour was attached to the hyoid, and prevented normal movement of the tongue, speech and swallowing. CT-scanning showed extension into the arytenoids. Submandibularly, separate lymph nodes were not palpable in the tumour mass, and the whole submandibular space was filled by the tumour (T_4, N_3, M_0).

The Karnofsky status was 40%, due to adenocarcinoma, recto-sigmoidectomy, and anus preternaturalis in 1979, TUR associated with prostatic hypertrophy in 1979, and a right tibial plateau fracture sustained in a traffic accident in 1982. A pressure sore on the right heel and cervical spondyloarthrosis were also present.

Because the size of the tumour made speech and swallowing practically impossible, and because the patient was highly motivated toward any possible cure, in spite of his poor general status it was decided to carry out bilateral retrograde cannulation of the superfical temporal artery, with local selective infusion of the right lingual, facial, internal maxillary and ascending pharyngeal arteries and the left lingual and ascending pharyngeal arteries.

Radiotherapy was concurrently administered with half dosage chemotherapy per catheter according to the treatment protocol (TD 4100 cGy). Tumour reduction was impressive. After 2 weeks, a severe mucositis (Grade IV, Appendix III) and dermatitis (Grade III, Appendix III) with a cutaneous fistula running toward the centre of the tumour, appeared.

Because of the severe mucositis, swallowing was still not possible directly after the combination treatment, despite the suppleness of the tongue and floor of the mouth. Recovery from local toxicity was lengthy (8 weeks), and was complicated by problems related to the pressure sores (resulting from leakage around the urinary catheter);

in the post-operative period a balano-posthitis also developed as a result of a paraphymosis, which necessitated a dorsal splitting of the prepuce.

After 2 weeks' combination treatment, the patient developed an aspiration broncho-pneumonia which responded well to treatment. Severe leucocytopenia and thrombocytopenia did not occur (Grade I, Appendix III). No problems were encountered with the catheters in this atherosclerotic man.

Immediately after discharge to a nursing home, there were problems with the pressure sores on hip, coccyx and heel; a naso-gastric tube was still used to provide adequate fluid and nutrition although swallowing was at this time no longer a problem.

A complete clinical response was seen to have occurred at follow-up. Tongue and mouth floor felt supple on manual palpation. The cutaneous fistulae had closed. The mucosa was atrophic over the whole intra-arterially perfused area. The OPG still showed a continuity defect of the horizontal ramus in the site where sequestrectomy had been performed during the combination treatment. Salvage surgery of the mandible and surrounding soft tissue was not considered appropriate for this weak patient.

In June 1983, a year after treatment, an abscess appeared in the region of the continuity defect of the mandible, with a fistula which extented extra-orally. Biopsies via the skin defect revealed tumour recurrence. Because of respiratory difficulty, a tracheostomy tube was placed. The patient was returned to his nursing institution a week later, and he died shortly afterwards from a purulent broncho-pneumonia. At post mortem, tumour recurrence was found in the mandible.

Case History 2.

Male, 76 years

The patient visited our clinic in May, 1982 with an extensive, poorly differentiated squamous cell carcinoma of the left maxilla. The whole maxillary sinus, floor of the nose, palate, maxillary tuberosity and orbital floor had been destroyed by the tumour. The CT-scan suggested parapharyngeal extension and spread beyond the medial pterygoid muscle. The skin of the left cheek could still be moved over a fixed swelling which extended from the nostril to the rostral border of the parotid gland. Intra-orally there was a swollen necrotic tumour mass of the cheek, pharynx and maxillary tuberosity extending just to the mid-line. Fixed lymph nodes (4cm) were palpable in the submandibular area and in the high cervical region (T_4,N_3,M_0).

The patient suffered from dyspnoea on effort, intermittent claudication complaints, tinnitus and uncertain gait. Having survived a heart attack he was using Sintrom. In 1981 an endarterectomy of the right femoral artery had been carried out at another institution. The Karnofsky status was 70%.

In the beginning of June, 1982, a radical neck dissection was performed under general anaesthesia with sacrifice of the accessory nerve in view of the large fixed mass of high cervical lymph nodes immediately ventral to the nerve.

Combination treatment was administered according to the protocol.

Radiotherapy consisted of high energy photons (8MeV) 2 plan parallel fields on the tumour area and on the whole neck 200 cGy, 5 fractions/week up to a TD of 4000 cGy. Similarly, 4000 cGy was administered via an AP field in 20 fractions to the supra-clavicular region. The total Adriamycin dosage over 5 weeks was 165 mg. The total Bleomycin dosage over 4 weeks was 120 mg.

After the 2nd week of combination therapy, there was a severe mucositis (Grade IV, Appendix III) in the target area, with a severe cheililtis and a Grade III dermatitis (Appendix III). A purulent keratoconjunctivitis also occurred. Large necrotic fragments were expelled from the central parts of the tumour. Because of pain with swallowing, a naso-gastric tube was introduced. The patient developed bronchopneumonia in the second concurrent combination therapy week, which responded well to treatment.

There were no problems with the arterial line. The Adriamycin was administered according to the protocol; a Grade I (Appendix III) leucocytopenia was seen. The dermato- mucositis was treated with antibiotics and wet bandages on the skin. After four weeks the skin and mucosa had healed well but a trismus had developed from the scar contraction in the tuberosity of maxilla and pterygoid muscle region.

In February, 1983 indications of lung metastases were found on follow-up chest radiographs. During the whole further follow-up period, no sign of local tumour was found either clinically, or in biopsies from the previous tumour region. The patient could eat and swallow normally. The patient refused a resection prosthesis for the defect in the maxilla.

Twelve months after termination of combination treatment, the patient died at home of bronchopneumonia. There was no evidence of local tumour recurrence. Post mortem examination was not permitted.

Case History 3.

Female, 51 years.

In August, 1982 the patient was referred to our clinic with a well differentiated carcinoma of the tongue and floor of the mouth. The tumour extended over the whole floor of the mouth from the right piriform sinus to beyond the mid-line on the left side. The tumour was attached to the mandible and was palpable in the base of the tongue extending over the mid-line. The submandibular lymph node complex appeared to be continuous with the primary tumour and was fixed to the underlying tissue. Similarly, upper cervical lymph nodes were also palpable and fixed to the underlying structures (T_4,N_3 ,N_0). Speech and swallowing were compromised. The general condition was good, and the Karnofsky index was 90%.

A radical en bloc cervical lymph node dissection was carried out in which it was not possible, because of the continuity of the primary tumour with the submandibular metastases, to obtain a tumour free dissection plane. During resection attempts in the submandibular area, the lingual artery was incised in the underlying musculature of the floor of the mouth, with the consequence that intra-arterial regional infusion from the lingual artery via retrograde cannulation, was no longer possible on the right side. The catheter was

introduced into the proximal end of the lingual artery (without flow) but lodged after 1 cm, in the tumour. The left side was cannulated routinely, retrograde via the superficial temporal artery.

During the first week of the treatment a leakage of the cytostatic agent in the operative area on the right side became apparent, after which the right arterial line was removed. The combination treatment was continued with a left arterial line and was seen as palliative. If sufficient tumour response had occurred after combination treatment (via the systemic circulation on the right side) together with radiotherapy, salvage surgery would still be a possibility. Because of the leakage of chemotherapeutic agent in the operative area, a right submandibular orocutaneous fistula developed.

In the second treatment week, the combination treatment had already caused a severe muco-dermatitis (Grade IV), and swallowing difficulties necessitated introduction of a naso-gastric tube. In view of the good clinical response of the tumour process, it was decided to continue with the combination treatment. During the therapy there were no further complications (e.g. severe reduction in thrombocyte and leucocyte counts (Grade I, Appendix III.), broncho-pneumonia).

The patient's recovery period was long because of the dermatitis and fistula in the right neck. Biopsies of the base of the tongue on the right side in September 1982 showed absence of vital tumour tissue. For this reason and in view of the large scale of possible salvage surgery, further treatment was not performed.

On follow-up examination 8.5 months after the negative biopsies, an ulcer of 1 cm diameter with a raised edge was found in the floor of the mouth and the biopsy revealed a moderately differentiated squamous cell carcinoma. On the insistence of patient and family, salvage surgery was however carried out (subtotal removal of the tongue, hyoid, cheek, pterygoid region, hemimandible, and half the oropharynx). Frozen sections of all resection surfaces showed no further tumour tissue. Reconstruction was carried out by means of a myocutaneous pectoral flap to reconstruct the internal surface and tongue, and a deltopectoral flap for the outer surface.

Pathological examination of the resection specimen showed a tumour mass in the whole glosso-alveolar groove; median section of the mandible showed that there was very little normal tissue peripheral to the tumour. All the other resection surfaces were free from tumour.

Wound healing after salvage surgery and reconstruction was successful. After 3 weeks swallowing was possible. The naso-gastric tube was however maintained because of the danger of aspiration. After 6 months the patient showed a recurrence in the pharynx behind the myocutaneous flap reconstruction. However, 6 further months elapsed before the patient, after protracted suffering, died of this recur- rence. Post mortem examination was not permitted.

Case History 4.

Male, 52 years

In August, 1982 the patient visited our clinic for examination of a well-differentiated carcinoma of the tongue and floor of the mouth, which probably originated in the right floor of the mouth and was

attached to the mandible. The tumour occupied both submandibular spaces and was growing via the base of the tongue in the floor of the mouth toward the contralateral side. The whole floor of the mouth from left to right was ulcerated. Caudal infiltration extended to the hyoid bone.

Multiple nodes were palpable (some larger than 2.5 cm): in the left and right submandibular triangle and the upper and middle deep jugular chain; none of the very large nodes was fixed to the common carotid artery (T_4 ,N_2,M_0). Swallowing and speech were painful and difficult. The Karnofsky index was 80%.

A radical neck dissection was carried out on the right side, as well as supra-omohyoid neck dissection on the left. However, histopathological examination of more than 50 nodes from all regions revealed no tumour tissue. Bilateral cannulation via the temporal artery was carried out so that local selective infusion of the lingual artery and left and right ascending pharyngeal arteries was possible. The whole visible tumour area was coloured when disulphine blue was infused.

Concurrent combination therapy was tolerated for only one week, after which dermatitis (Grade IV) developed in the perfused area, together with cutaneous fistulas under the chin. This was probably the result of leakage of cytostatic agent from the wound area to the skin.

It was decided to exclude the neck from the radiation area (partly because of the histopathological findings of the neck dissection specimen) and to continue the treatment at half dosage of the concurrent combination therapy. The radiation therapy was terminated after 2000 cGy. The patient did receive Bleomycin during the third weekend, according to the protocol. The patient's condition deteriorated slowly. A fever peak was initially attributed to the Bleomycin administration of the previous night because the lungs did not reveal any abnormality on auscultation or radiological examination. The severe mucositis (Grade IV) made swallowing very difficult. A leucopenia (Grade I) developed. The patient was given corticosteroid and broad spectrum antibiotic medication. In the evening, the temperature rose above 40 C. The patient went into irreversible toxic shock, and died several hours later. The cause of death was massive sepsis. Cultures of Klebsiella, E. coli, Enterococcus, and Staphylococcus aureus were obtained from the heart, spleen, lungs and blood. At the time of death the patient had received half of the combination treatment. At post mortem, histopathological examination of the tongue and floor of the mouth demonstrated the presence of tumour tissue.

Case History 5.

Female, 35 years.

In November, 1982 the patient was referred to our clinic with a moderate to well-differentiated squamous cell carcinoma, probably originating in the floor of the mouth on the left side with spreading to the faucial arch, tonsil, left pharyngeal wall, base of the tongue, retromolar maxillary tuberosity, and soft palate. The tumour extended along the soft palate to the mid-line. In the submandibular triangle and upper and mid cervical regions, lymph nodes were palpable (T_4 ,N_1 ,M_0). There was difficulty in swallowing. The Karnofsky index was 80%.

Two weeks after admission, a radical cervical node dissection was carried out. The superior temporal artery was retrogradely cannulated with ligation of the vessels not perfusing the tumour (the occipital artery, the facial artery and the temporal artery). The neck dissection specimen showed that only 2 of the 27 large nodes contained tumour and these were situated in the high cervical and submandibular regions.

Combination radiation treatment was tolerated well, in spite of the muco-dermatitis (respectively Grade IV and III, Appendix III). Severe leucopenia and thrombocytopenia did not occur (Grade I, Appendix III), and there were no problems with the catheter. In the light of the patient's relatively high tolerance of the treatment, 5 weeks of concurrent combination treatment were completed (total dosage 5000 cGy). Recovery from the mucositis was successful. There was no indication of the presence of local regional tumour.

In May, 1983, multiple biopsies were taken under general anaesthesia in the original tumour region. No vital tumour tissue was found. The patient functioned normally within her family although smoking 20 cigarettes per day. She was tumour-free at the last follow-up.

Case History 6.

Female, 78 years

In April, 1983, the patient was referred to our clinic with a well-differentiated squamous cell carcinoma of the left maxilla. The tumour extended caudally to the faucial arch and retromolar trigone; buccally, the tumour extended into the cheek. The mid-line was not clinically invaded and the orbita appeared to be tumour-free. Resectability in the parapharyngeal region seemed dubious because of infiltration of tumour into the pterygoid musculature, and pterygoid plates. There were some small (0 - 1 cm) lymph nodules palpable in the ipsilateral submandibular and high jugular regions of the neck (T_4 ,N_1 ,M_0). The patient had a 1st degree atrio-ventricular block and fibrillation, migraine attacks, and had been a victim of epilepsy attacks in the past. The Karnofsky index was 80%.

After a standard radical neck dissection had been carried out with sparing of the cranial branch of the accessory nerve, retrograde cannulation of the superfical temporal artery was attempted through a pre-auricular approach. The vessel was very sclerotic and ran behind the temporo-mandibular joint in such a tortuous fashion that this access was abandoned. The catheter was retrogradely introduced via the occipital artery and was advanced into the external carotid with the tip exactly above the superior thyroid artery. The lingual and facial arteries were ligated.

The patient was digitalized post-operatively, due to fibrillation. Concurrent combination treatment ran according to the protocol. Already in the second week, local mucositis (Grade III, Appendix III) and swallowing difficulties had developed and naso-gastric tube feeding was necessary.

Problems with the intra-arterial infusion appeared at the end of the third combination treatment week. Leakage of the cytostatic agent occurred at the catheter insertion site; after the introduction of new ligatures, the combination treatment could be completed.

Complete clinical remission was confirmed after the termination of the therapy. In the recovery phase of the dermato-mucositis, the patient developed broncho-pneumonia, probably because of aspiration. There was a good response to therapy, the temperature dropped and the blood gas values became normal. In spite of effective digitalization, the patient developed cardiac decompensation with pulmonary oedema. Chest radiograpghs showed diffuse patches of infiltrative lesions and pleural fluid. The patient was transferred to the internal medicine department. After intensive treatment there, the acute picture resolved after 5 days. After a gradual improvement of the general condition, the patient again became feverish and there were indications of ileus. In spite of instigation of an appropriate therapy, the patient's condition deteriorated quickly and she died shortly afterward.

There were no indications of local regional tumour. Post mortem examination was not permitted.

Case History 7.

Male, 78 years.

In May, 1983, a partial tongue resection had been carried out at another hospital, because of a well-differentiated squamous cell carcinoma of the left edge of the tongue. Five months later he came to our clinic with a local recurrence.

From staging under general anaesthesia and from the CT-scan, it could be seen that there was infiltration in the base of the tongue to the hyoid, floor of the mouth and mandible. The palpable swelling extended dorsally to the piriform sinus. Ipsilateral nodes were palpable sub-mandibularly and subdigastrically (T_4 ,N_1 ,M_0). The patient was in pain and had difficulties with swallowing and speech. The Karnofsky index was 60%, and associated with a previous heart infarct, a first degree atrio-ventricular block and hypertension were present.

After a standard neck dissection (May, 1983) with sacrifice of the cranial branch of the accessory nerve due to the presence of positive nodes in the high jugular area and spinal accessory chain, retrograde cannulation via the superficial temporal artery was performed. The vessels which did not perfuse the tumour region (the facial artery and the occipital artery) were ligated and a ligature was placed cranial to the catheter tip. The tumour region stained well despite the prior surgery. The enlarged nodes in the neck dissection specimen contained carcinoma without capsule infiltration.

During the combination treatment 4000 cGy was administered in 19 fractions to the primary tumour region and the neck. During treatment, the patient developed a mucositis (Grade IV) in the perfused area with increasing pain and discomfort. Complete clinical remission occurred.

After 3 months further biopsies were taken from the original tumour area and these contained only collagen-rich connective tissue with non-specific inflammatory infiltrate. Tumour tissue was not found. At subsequent follow-up, the patient remained free of local regional tumour. Nine months after treatment, the patient died at home of heart failure. Post-mortem examination was not permitted.

Case History 8.

Male, 61 years.

The patient was referred to our clinic in June, 1983 for examination of a squamous cell carcinoma of the left tongue and floor of the mouth, with extension over the mid-line in the base of the tongue and infiltration pharyngeally into the pre-vertebral fascia. The ulcer could be seen to extend to the pharyngo-epiglottic fold. Lymph nodes were palpable in the left and right subdigastric region (2 cm) and right submandibular (1 cm) (T_4,N_2,M_0). Swallowing was difficult and the patient choked frequently. He had pain in the tongue which extended to the ear. The Karnofsky index was 70%.

A left-sided cervical node dissection was carried out leaving the accessory nerve intact because the frozen sections of the cervical nodes from the high cervical area showed no evidence of tumour. A catheter was introduced via the temporal artery to just beyond the branching of the lingual artery. The occipital, thyroid, and facial arteries were ligated. At the same time a ligature was applied cranial to the catheter tip to restrict the flow to the lingual artery and the ascending pharyngeal artery. On the right side the large nodes along the internal jugular vein were also shown to be tumour-free in the frozen sections. The superficial temporal artery on the right side was then cannulated. The tongue and the pharynx were stained on both sides after infusion of disulphine blue.

Because catheterization was bilaterally performed, the doses of Adriamycin and Bleomycin per catheter were reduced by half. Thus an equal amount of the cytostatic agent was delivered to the target area; the flow from the catheter tip was however as low as possible due to the selective ligation. Radiotherapy consisted of 8 MeV photons in 2 plan parallel fields in 25 fractions of 200 cGy (total dose 5000 cGy) delivered to the neck and the primary tumour.

The simultaneous combination treatment followed the protocol until the end of the third week, by which time a moderate dermatitis (Grade I) and mucositis (Grade IV) of the tongue had occurred, which necessitated naso-gastric tube feeding. At the end of the third week, the patient developed a high temperature peak and despite antibiotic therapy, the patient maintained this high temperature. Both catheters were therefore removed after which the temperature dropped precipitously (blood cultures and catheter tip cultures were later shown to be positive).

After a 2 week rest period, the catheters were again introduced, this time via the occipital artery. During anaesthesia, a hard palpable infiltrate was detected at the base of the tongue, the ulcer had completely disappeared and the mobile part of the tongue was supple. A biopsy taken from the base of the tongue contained only haemorrhagically altered tissue with radiation effects; vital tumour tissue could not be demonstrated (N.B. after treatment with 2400 cGy, 90 mg Adriamycin and 90 mg Bleomycin). The combination treatment was continued up to a total dosage of 5000 cGy.

In the fourth therapy week, a cutaneous defect became apparent on the left side in the distal part of the upper McFee incision (there was however, no leakage of the chemotherapeutic agent). The patient's recovery was slow. The skin healed well, but swallowing remained difficult and the patient would not be without the naso-gastric tube. As he was alone, he could not manage at home and was admitted to a

nursing home in his own neighbourhood. Six months later, he developed a skin defect on the left side which was closed using a deltopectoral flap. Under general anaesthesia, biopsies were taken from the tongue base and floor of the mouth and no tumour tissue was found histologically. The skin defect recurred however, and the patient was not able to live without the naso-gastric tube until the time of his death eight months after completing the concurrent combination therapy, probably from broncho-pneumonia. Post-mortem examination was not permitted.

Case History 9.

Male, 77 years.

The patient was referred to our clinic in March, 1983, with a pathological fracture of the mandible at the level of the left mandibular angle, associated with a well-differentiated squamous cell carcinoma originating in the left retromolar trigone. The extension into soft tissue was lingually to the base of the tongue, distally to the posterior pharyngal wall, and buccally from the mandible to the parotid gland. Multiple ipsilateral lymph nodes (larger than 2 cm) were palpable submandibularly and subdigastrically (T_4, N_1, M_0). The Karnofsky index was 80%.

The patient had a history of TIA's and left-sided claudication complaints. A subclavian steal syndrome was diagnosed. This is not of haemodynamic significance in cannulation of the left external carotid artery.

In April, 1983, a standard cervical lymph node dissection was performed. The whole tumour mass above the hypoglossal nerve was left intact, probably still containing submandibular nodes. Attempts at retrograde cannulation of the superior temporal artery were unsuccessful. Cannulation was only successful up to the "rideau stylien" (6 cm). Careful manipulation of the head and rotation of the catheter did not allow further advancement. In order to avoid the danger of perforation (in the tumour), cannulation of the occipital artery was opted for. This was successful and the whole tumour area stained blue. The external carotid artery was not ligated at the level of the occipital artery so that the alveolar mandibular artery could be perfused through the internal maxillary artery in order to realize the benefits of perfusion of the central parts of the mandible. (This does allow a much greater flow at the catheter tip). Examination of the dissection specimen demonstrated only reactive lymph nodes.

The combination treatment resulted fairly quickly in mucositis and dermatitiis (respectively Grade IV and III, Appendix III). Bleomycin infusion caused high temperatures and shivering. After 2 weeks of the combination treatment, tumour regression was difficult to evaluate because of local infiltrate and oedema at the angle of the jaw.

The combination treatment was continued with difficulties (pain, mucositis (Grade IV), choking, high temperature after Bleomycin administration, moderate leucocytopenia (Grade I), and completed up to the end of the fourth concurrent combination treatment week. The final dose of Adriamycin was not administered because of problems with the catheter (pain on flushing, probably as a result of a thrombus). Recovery was long (6 weeks). Further follow-up was delayed too long; in the light of present knowledge, salvage surgery

(mandible resection) should have been considered directly after the immediate post-treatment period (in spite of complete clinical remission) because of massive central bone infiltration in the mandible, and the absence of a clear increase of tumour cells in the G_2+M-phase of the cell cycle during combination treatment.

Five months after completing the combination therapy the patient developed an ulcer on the alveolar process in the left molar region. Salvage surgery followed. Frozen sections in all the resection boundaries were tumour-free. Histopathological examination of the resection specimens showed however that the resection surfaces in the medio-ventral tongue musculature were not tumour-free. For this reason, a repeat resection was immediately carried out of tongue and floor of the mouth and the mandibular resection stump on the contralateral side.

Recovery after the second surgical intervention was successful. The patient could swallow and speak, and did not require permanent naso-gastric entubation. Four months later, we diagnosed contralateral submandibular skin metastases and a carcinomatous lymphangitis. The patient died 4 months later of extensive local tumour recurrence.

Case History 10.

Female, 72 years.

In July, 1983, the patient was referred to our clinic with a right-sided tongue and floor of the mouth carcinoma with extensive bone invasion and a pathological mandible fracture. The whole right submandibular area was occupied by tumour, which had adhered to the mandible.

Staging under general anaesthesia (necessitated by trismus) showed infiltrative growth into the tongue over the mid-line with an ulcer extending to the piriform sinus and in the rostral part of the faucial arch. The CT-scan showed infiltration in the base of the tongue to the hyoid, but parapharyngeal spread was difficult to evaluate. In the mid cervical area, fixed lymph nodes (larger than 3 cm) were palpable (T_4,N_3,M_0). The Karnofsky Index was 70%.

A cervical lymph node dissection was carried out, and a large fixed upper jugular lymph node could be dissected from the carotid artery (histological examination showed infiltration into the jugular vein and through the capsule) and at the same time mid-jugular nodes appeared to contain tumour. On cannulation of the superficial temporal artery, the catheter contacted the tumour after about 6 cm. The occipital artery was then cannulated. The whole tumour area and the suprahyoidal region stained well with disulphine blue.

The first half of the concurrent combination treatment was tolerated well. At this stage, no more tumour was clinically evident. Mucositis and dermatitis had in the mean time appeared (respectively Grade IV and III, Appendix III). It was decided to continue the treatment. The last part of the concurrent combination treatment presented many compli- cations, such as broncho-pneumonia, fever after Bleomycin administration, severe muco-dermatitis and dysphagia.

The recovery period was long. Clinical remission appeared to be complete. Radiologically, a continuity defect of the mandible, with

smooth edges, remained. Four months after completing the treatment, an ulcer developed on the alveolar process at the site of the mandibular defect. Biopsies revealed tumour recurrence. Salvage surgery was performed. Frozen sections were made of all the resection boundaries and no tumour tissue was found. On examination of the resection specimen, the boundaries were also found to be tumour-free. During the follow-up, the patient progressed slowly. Return to her home was not possible and she was admitted to a nursing home.

In March, 1984 (4 months after the last surgical inter- vention) there was a second recurrence in the submandibular skin. There was every reason to abandon further treatment, but the patient and family could not be dissuaded. For this reason, further surgery was carried out. Reconstruction of the defect followed by way of a myocutaneous pectoral flap on the inside, and a deltopectoral flap on the outer surface. The resection boundaries were all, once more, negative. Cure was successful and the recovery went well. A naso-gastric tube continued to be necessary.

After 4 months, metastases were again visible, this time low in the neck on the contralateral side. The aspect was that of a carcinomatous lymphangitis. The patient died six months later of extensive local metastases.

Case History 11.

Male, 49 years.

In September, 1983, the patient was referred to our clinic with a moderately to well-differentiated squamous cell carcinoma of the left maxilla. The extension from the ulcer ran from the hard palate via the soft palate to the left faucial arch. Radiological bone lesions were evident from the canine region to the maxillary tuberosity. The spread was visible just over the mid-line in the region of the A-line. Infiltration via the retromolar trigone and the tonsillar region appeared to extend to the mid-parapharyngeal region and the pterygoid muscle. Subdigastrically, on the left side, a 5 cm node was almost continuous with the tumour and fixed to the underlying tissue (T_4 ,N_3 ,M_0). The patient was in pain and had, a short time previously, experienced bleeding from the pharynx. The Karnofsky Index was 80%.

In the neck dissection carried out, the lymph nodes could be dissected from the external carotid artery. There was histological evidence of central necrosis, capsule penetration and growth into the jugular vein.

Retrograde cannulation of the superficial temporal artery failed, and therefore retrograde cannulation via the facial artery was performed. The occipital and the superficial temporal arteries were ligated. The whole tumour region and the neck in the region of the dissected subdigastric node stained well with disulphine blue.

The combination treatment which went according to the protocol was well tolerated by the patient. There were no problems with the catheter. The combined radiation, consisting of 2 plan parallel fields and 4000 cGy, was administered in 20 fractions to the whole tumour process and the neck. A Grade IV mucositis developed which prevented continuation of the combination treatment into the fifth week. The primary tumour was clinically in complete remission.

Recovery from the mucositis took approximately 4 weeks. After a follow-up period of 4 months, an oro-nasal fistula developed centrally in the hard palate at the site where the tumour had been observed crossing the mid-line at the first staging. For this reason, salvage surgery was indicated, and a sub-total maxillectomy together with resection of the soft palate, pharynx, and the retromaxillary area on the left side (the primary tumour region) was performed.

The frozen sections showed that the tumour on the contralateral side already demonstrated infiltration beyond the pterygoid musculature. Consequently, a repeat resection was carried out at the same time, involving the whole parapharyngeal region to the base of the skull, leaving the right internal jugular vein and the internal carotid artery intact.

For the description of the histopathological examination of the resection specimen, see Chapter VII, 3.2.

A resection prosthesis was constructed and the patient was discharged in a reasonable condition. Three months later, there was tumour recurrence on the right side (contralateral to the primary tumour) in the glosso-alveolar groove continuous with the resection boundary. It was decided to administer a "palliative" methotrexate therapy according to the Kirkwood protocol (1981): Methotrexate 50mg/m on days 1 and 4, Leucovorine rescue 50mg/m on days 2 and 5.

Under this treatment, the tumour remained stationary over a 4 month period, but the general condition of the patient deteriorated slowly. Six months after the salvage surgery, the patient died with a tumour recurrence in the right neck and pharynx. The left neck (the side with the fixed nodes) was found, at post mortem, to be tumour-free,

Case History 12.

Male, 51 years.

In December, 1983, the patient was referred to our clinic for examination of a moderate to well-differentiated squamous cell carcinoma of the right tonsillar region with extension into the parapharyngeal area, and attachment to the posterior pharyngeal wall, retromolar trigone, and floor of the mouth. The CT-scan showed infiltration into the pre-vertebral fascia. Sub-mandibularly a small node was palpated (smaller than 1cm) (T_4,N_1,M_0). The patient had survived a heart infarct (antero-septal) two years earlier, had undergone aorta bifurcation bypass prosthesis surgery one year earlier, and complained of angina pectoris and varicosis. The Karnofsky Index was 80%.

A radical neck dissection was performed, preserving the accessory nerve. The internal jugular vein was completely thrombosed. In the dissection specimen only reactive lymph nodes were later diagnosed. Cannulation of the superficial temporal artery was successful in this sclerotic patient. The occipital artery was ligated. Disulphine blue staining of the whole tumour area was possible.

During the first week of combination treatment, there were no complications. However in the second week, the patient experienced pain after infusion of approximately half of the chemotherapy dosage (30 ml = 7.5 mg Adriamycin in 4 hours). Disulphine blue staining of the tumour area was achieved (this procedure was however, also

painful), and for this reason chemotherapy was continued. With administration of the following dosage, nocturnal pain and a heavy feeling in the target area was experienced. The following day angiograms were made of the common carotid artery. A total occlusion of the external carotid artery from the point of bifurcation was found. The areas surrounding the contrast injection along the catheter showed filling of the lingual, internal maxillary, and ascending pharyngeal arteries. Disulphine blue staining of the tumour was thus, via the retrograde route, still possible in spite of the occlusive thrombus of the external carotid artery. The chemotherapeutic agent should then, however, be transported to the tumour region without blood flow, which increases the intra-arterial infusion effect, but is too dangerous with respect to possible occurrence of vessel wall necrosis of the perfused vessels. It was decided to remove the arterial line after half of the combination treatment (2 weeks' combination therapy). In view of the almost complete tumour regression, it was decided to continue the radiotherapy to 4000 cGy (second part of the combination treatment without chemo- therapy).

The mucositis was clearly less severe than in other patients in this study and the recovery period was shorter. At the end of this treatment, a complete clinical remission had occurred. It was decided to go ahead with salvage surgery of the whole previous tumour area because in the last two weeks of combination treatment no chemotherapy had been given. At the end of February, 1984, block resection of the mandible, caudal pharyngeal wall, part of the tongue, tonsillar area, inner cheek surface and tuberosity of maxilla was performed.

The frozen sections of the resection boundaries showed no tumour tissue. The defect was reconstructed by way of a myocutaneous pectoral flap. Wound healing was successful. In the resection specimen, multinuclear giant cells around keratin fragments were found, but there was no vital tumour tissue detected. The patient at the time of this report is tumour-free and has returned to work.

Case History 13.

Male, 60 years.

In May, 1984, the patient was referred to us with a well-differentiated squamous cell carcinoma, probably originating in the right retromolar trigone, with spread to the soft palate (over the mid-line), maxillary tuberosity, cheek, parapharyngeal region, with attachment to the posterior pharyngeal wall, floor of the mouth, base of the tongue, and the mobile tongue. Ipsilaterally, cervical lymph node metastases were palpable, in the upper cervical region (larger than 3 cm) attached to underlying tissues, and in the submandibular triangle (1 cm) and mid-cervical (1 cm) regions (T_4,N_1 ,M_0). The Karnofsky Index was 80%.

An ipsilateral radical neck dissection was carried out with sacrifice of the accessory nerve in view of the localization and the aspect of the nodes. Retrograde cannulation of the superficial temporal artery was carried out. Only the occipital artery was ligated. The tumour was localized in the perfusion area of all the other external branches. At the same time the superficial temporal artery on the left side was cannulated, and the facial and occipital arteries were ligated. Histopathological examination of the neck dissection specimen revealed capsular infiltration of the nodes with carcinoma

and ingrowth into the internal jugular vein.

The first two weeks of combination treatment were well tolerated. Tumour regression was greater than 50%. Mucositis and dermatitis (respectively Grade IV and III, Appendix III) developed and necessitated the patient being fed by naso-gastric tube. At the end of the fourth concurrent combination treatment week, the treatment was terminated after 3800 cGy in 19 fractions had been administered.

Recovery after combination treatment was long (5 weeks). In the light of experience with extension of the primary tumour in the mandible, it was decided to carry out resection of all the osseous structures and soft tissue situated in the primary tumour area immediately after recovery from the concurrent treatment.

No tumour was found in the frozen sections of any of the resection surfaces, and the histologically examined resection specimen was also found to be tumour-free. Reconstruction of the resection cavity followed using pharyngoplasty and a resection prosthesis. The patient functioned again in his previous work situation, and consumes 5 cigars a day and considerable quantities of alcohol.

Case History 14.

Male, 50 years

In August, 1984 the patient was referred to us with a moderately differentiated squamous cell carcinoma which had destroyed the whole maxillary sinus on the right side, with infiltration into the ethmoid, pterygoid and the orbit. The tumour was necrotic in the centre. There was a large naso-oro-antral fistula. The defect extended well over the mid-line and was attached to the skin of the right cheek. The CT-scan revealed bone destruction of the whole maxillary tuberosity (involvement of the skull) and invasion of the carcinoma into the pterygoid muscles. Upper and mid cervical lymph nodes (2 cm) and submandibular lymph nodes (2.5 cm) were palpated (T_4, N_1, M_0). Speech and swallowing had become difficult and the patient was in great pain. The Karnofsky Index was 80%.

A radical cervical node dissection was carried out on the right side. The accessory nerve was left intact. Retrograde cannulation of the superficial temporal artery was successful, probably through the tumour, in which the vessel wall was sufficiently intact to allow manoeuvering of the catheter. Selective perfusion was achieved by ligation of the lingual and occipital arteries. Contralateral cannulation was carried out at the same time via the superficial temporal artery so that the whole maxilla could be regionally perfused.

The combination treatment resulted at an early stage in a severe muco-dermatitis (respectively Grade IV and III, Appendix III) of the whole infused area. In view of the favourable tumour regression, it was decided, in spite of the severe mucositis and purulent dermatitis, to complete the full combination treatment. In the fourth and last combination therapy week, chemotherapy was reduced (50% dosage = 7.5 mg Adriamycin) because of regional toxicity. The presence of general toxicity was indicated by a leucopenia (Grade I, Appendix III). Besides several fever incidents, probably associated with the oral infection, the patient sustained a broncho-pneumonia, which responded to the therapy instituted.

The left (contralateral) arterial line caused pain during the infusion of chemotherapeutic drugs in the third treatment week. Staining of the left maxilla was no longer apparent after disulphine blue infusion. Angiography demon- strated a complete obstruction of the external carotid artery above the branching of the occipital artery (the facial, lingual and occipital arteries were still supplied with blood from the external carotid). Retrograde contrast injection demonstrated the presence of an extravasation cavity, which necessitated removal of the left catheter in the third week of combination therapy.

The recovery period was long (6 weeks). Because of the massive bone invasion and the premature removal of the left catheter, resection of the initial tumour area was decided upon, despite complete tumour regression. In January 1985 block resection, involving subtotal maxillectomy and removal of the orbital floor and ethmoid, was carried out. Frozen sections of the resection surfaces did not demonstrate tumour tissue. The resection specimen contained vital tumour tissue in the centre of the initial tumour area at the site over the mid-line which did not receive the full chemotherapy dose in concurrent combination therapy due to the early removal of the left arterial catheter. A resection prosthesis was made. The patient has returned to normal domestic life, but unfortunately still smokes many large cigars each day.

In February 1986, a recurrence in the resection plane at the base of the skull was detected 19 months after combination therapy had begun and three months later he died.

Case History 15.

Male, 68 years.

In January 1985 the patient was referred to our clinic for an examination of a well-differentiated squamous cell carcinoma of the right maxilla, with extension to the ethmoid, orbit, retromaxillary area, and the cheek. The tumour crossed the mid-line at the junction of the hard and soft palates. Obvious proptosis was present. A 2cm lymph node, was present in the mid-cervical region (T_4,N_1,M_0). The patient had symptoms of COPD and Parkinson's disease. The Karnofsky Index was 70%.

A radical cervical node dissection was performed on the right side and the accessory nerve spared. After retrograde cannulation of the right superficial temporal artery the tumour region was selectively perfused, the tip lying immediately below the maxillary artery. The same procedure was conducted on the left side because of extension of the tumour over the mid-line.

The administration of the first dosage of Bleomycin was associated with fever (> 41 C), shivering, anxiety and confusion, and exascerbation of the Parkinson symptoms. The neurological signs disappeared quickly and the fever dropped after withdrawal of Bleomycin. Because of the neurological side-effects and the COPD, it was decided not to administer Bleomycin again.

In the first two weeks of the combined treatment, large-scale tumour regression occurred. At the site of the original carcinoma, a large defect in the maxilla containing necrotic debris was present. A Grade III mucositis was evident.

In the third week of concurrent combined treatment, the patient experienced pain during flushing of the left arterial line. Disulphine blue infusion no longer stained the left palate. Arteriography of the left common carotid showed complete obstruction of the left maxillary artery and the left arterial line was therefore removed. Because of experiences with other patients (10 and 14), it was decided to perform salvage surgery at the termination of combined treatment. Combined treatment was otherwise successful with the right arterial line (the side of the primary tumour). Despite the increased dosage of Adriamycin, the systemic toxicity was mild as evidenced by a leucopenia which never exceeded Grade I (Appendix III). Halfway through the last (fourth) combined treatment week, severe pain and swallowing difficulties were experienced, and the patient was fed through a naso-gastric tube. There was a peracute rise in body temperature. Blood was taken for blood gas analysis and microbiological culture (the latter remained negative). Thoracic radiography showed no lesions except of the pre-existent COPD. Despite the institution of antibiotic therapy, the patient died some hours later, apparently of endotoxaemic septic shock.

At post mortem there was evidence of pulmonary oedema and cardiac dilatation. In the trachea and primary bronchi purulent froth was found. Microscopically there was no indication of myocardial changes attributable to Adriamycin. The cause of death was thus probably (aspiration) pneumonia resulting from the severe mucositis and necrosis in the tumour area caused by the concurrent combined therapy. Vital tumour tissue was demonstrated in the post mortem maxilla specimen (i.e. after 3 weeks' concurrent therapy with Adriamycin and radiotherapy.)

Case History 16.

Female, 54 years.

The patient was referred to our clinic in January, 1985 with a carcinoma of the right base of the tongue and floor of the mouth with extension over the mid-line. The tumour extended to the posterior pharyngeal wall and the right faucial arch and soft palate.

The submandibular area was completely occupied by a metastatic tumour process (larger than 10 cm), and lymph nodes (larger than 3 cm) were palpable in the upper cervical region (T_4 ,N_3,M_0). The patient experienced problems with swallowing. The Karnofsky Index was 80%.

A radical cervical dissection was performed, and the distal buccal part of the tumour appeared to be continuous in the hyoid region with the massive submandibular lymph node metastasis. The superficial temporal artery was cannulated ipsilaterally (on the right) and the catheter advanced to the superior thyroid artery. Because of the extent of the tumour process, only the occipital and facial arteries (at the border of the mandible) were ligated. On the contralateral side, the superficial temporal artery was cannulated, and the intra-arterial perfusion area was limited to the lingual and ascending pharyngeal arteries by ligation of the other external carotid branches. Biopsy for DNA flowcytometry was severely hindered by strong reflex activity during inspection of the tongue and floor of the mouth.

The first two weeks of combined treatment (total radiotherapy dosage 2000 cGy) was tolerated well. In the third week, the tongue no longer stained on the contralateral (left) side and Adriamycin

infusion caused pain. At the same time an ipsilateral (right) orocutaneous fistula developed, which stained blue after disulphine blue administration. On Seldinger aortic arteriography, the whole left external carotid artery was no longer orthogradely perfused, with the exception of the superior thyroid artery and several small branches of the facial artery at the origin of the branching from the external carotid. This finding necessitated removal of catheters from both sides at the end of the third combined treatment week. Radiotherapy was continued up to a total dosage of 5000 cGy.

Massive necrosis of the tumour and severe mucositis (Grade III and IV) during the therapy caused dysphagia and pain. The patient also sustained a broncho-pneumonia which responded to the instituted treatment. Following recovery from the mucositis, biopsies were taken under general anaesthesia from all regions of the previous tumour. Tumour tissue was not evident on histopathological examination. Radiotherapy was subsequently increased to 6000 cGy by administration of 5 further fractions.

The orocutaneous fistulas at the angle of the jaw and hyoid were closed by the use of a deltopectoral flap. Recovery of swallowing function followed succeasfully. The patient is, at the time of this report, able to speak and swallow normally. The floor of the mouth and tongue are supple but the mucosa is atrophic over the whole intra-arterially perfused area.

CHAPTER XI

RESULTS

1. Survival.
2. Causes of Recurrence.
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1. Survival.

Sixteen patients with non-resectable oral cavity carcinoma diagnosed at first admission were treated with combination chemotherapy and radiotherapy as described in Chapter X.

Four of the 16 patients are alive with 'no evidence of disease' (A - N.E.D.) after short-term follow-up (Fig. 9, Group A.).

Three patients died with 'no evidence of disease' (D - N.E.D.) (Fig. 9, Group B).

In six patients there was a tumour recurrence (R.E.C.) (Fig. 9, Group C).

Two patients died during the course of therapy (Fig. 9, Group D), one due to effects of irreversible toxic shock, the other from broncho-pneumonia.
Another patient died soon after therapy of intercurrent disease, clinically free at this time.

Subsequent surgery after combination therapy followed in three patients (Cases 12, 13 and 14, Fig. 9), in two cases no tumour tissue could be found in the resection specimen.

Figure 9. Survival and Follow-up

	Case No.	Follow up in months	Surgery after comb. CHT + RXT: Specimen free of Tumour -	Specimen with Tumour +	Salvage Surgery after Recurrence
Group A	5	42			
Alive - No Evidence	12	30	0		
of Disease (A-N.E.D.)	13	22	0		
	16	17			
Group B	2	12			
Dead - No Evidence	7	9			
of Disease (D-N.E.D.)	8	8			
Group C	1	12			
Loco-regional	3	8		+	2nd recurrence after surgery
Recurrence (REC)	9	5		+	
	10	4		+	
	11	4		+	
	14	19		+	
Group D					
Dead with Disease	4	no follow up			
during therapy	15	no follow up			
(D.W.D)					
Dead soon after therapy	6	no follow up.			

Four patients underwent salvage surgery (Cases 3, 9, 10 and 11) after manifestation of local recurrence. In all of these cases there was a second recurrence.

In 7 of the 16 patients (44%) (Fig. 9, Group A and B), no evidence of tumour was obseerved after short-term follow-up (A - N.E.D. and D - N.E.D.). These patients were spared death from the effects of the locoregional tumour process despite non-resectability of the carcinoma at the time of initial staging.

In spite of the 100% complete clinical remission in 14 patients, recurrence occurred in 6 of them.

2. Causes of Recurrence.

In 2 of the 5 patients who died from the effects of local tumour recurrence, the actual intra-arterial chemotherapy had not taken place (Cases 3 and 11).

In one case in which the lingual artery was incised during the neck dissection, the contralateral lingual artery was used to intra-arterially perfuse the contralateral part of the tumour although the greater part of the tumour was perfused only after systemic recirculation of the chemotherapeutic agent.

In the other case, there was a recurrence just over the mid-line of the hard palate, i.e. recurrence occurred in the systemically treated side. In this patient, the chance of cure would have been greater using bilateral infusion.

3. Complications and Errors of Concurrent Combined Therapy.

3.1. Intra-arterial Infusion Technique

Of the 26 cannulations performed in the 16 patients, the superficial temporal artery was cannulated on 20 occasions, the occipital artery 5 times, and the facial artery once. All cannulations were retrograde.

3.1.1. Serious Complications Related to Intra-arterial Catheter Technique.

a. In 5 of the 16 patients, it was necessary to remove the catheter prematurely, with the result that combined therapy to the desired dosage could not be completed. In all cases the reason for catheter removal was thrombosis in the artery downstream from the catheter tip. Four of these 5 patients were catheterized bilaterally, and in all 4 the contralateral catheter had to be removed. In 2 cases the treatment could

be completed through the ipsilateral catheter, i.e. on the side of the primary tumour and the neck dissection.

b. Neither temporary nor permanent cerebral complications were observed in the patients studied. Freckman (1972) recorded 8% temporary, and 6% permanent cerebral complications in his patients, as well as air embolus as described by Jesse et al. (1964) and Szabo and Kovacs (1979). We did not observe such complications in our study because firstly the catheters were always retrogradely introduced through the temporal, occipital or facial artery. Cerebral complications are clearly correlated with the mode of cannulation, and are least common when retrograde catheterization is employed (Espiner, 1962; Sullivan, 1962; Snow, 1966). Secondly, the catheter tip was positioned sufficiently far from the bifurcation to prevent flow of the cytostatic agent into the internal carotid artery.
Cerebral complications occurring early in the treatment period are usually of vascular origin, and of a temporary nature if the position of the catheter is corrected (Snow, 1966). Cerebral complications in the later stages of treatment are usually associated with thromboses in the area of the carotid bifurcation and subsequent embolization. These hemiplegias or hemipareses are usually permanent. Patients with arteriosclerotically changed vessels are predisposed.
Summarizing, it can be said that cerebral complications associated with selective intra-arterial infusion of the external carotid artery can generally be prevented if retrograde cannulation is employed, careful checking of the catheter tip position at regular intervals is carried out, good anticoagulation methods against catheter obstruction are applied (heparin and Ascal), and if the intra-arterial catheter is fitted with a permanent "flush system".

c. Extravasation of the cytostatic agent along the catheter at the site of entry in the cannulated vessel occurred in one patient with a catheter positioned such that the vessel was closed (following lingual artery incision in Case 3), and in three patients towards the end of the combined treatment.
The local submandibular and high cervical necrosis caused oro-cutaneous fistulas, mostly after thrombosis of the target area artery. In three of the four patients, the oro-cutaneous fistulas were closed with local pedicle flaps. One patient sustained recurrence of the fistula, and in one patient spontaneous closure of the oro-cutaneous fistulous tract occurred.

3.1.2. Minor Complications.

a. Dislocation of the catheter tip by direct traction on the catheter occurred in one patient, who constantly drew the catheter cranially when putting on her reading glasses.

Disulphine blue injection did not result in staining of the target area. The catheter was repositioned several times before the cause of dislocation was detected.

b. Loosening of the cranial ligature of the catheter tip decreased the selectivity of intra-arterial perfusion (this also occurred in the patient mentioned in a.). The cause was probably necrosis of the vessel wall at the ligature site. Another plausible possibility is traction on the catheter by flexion and extension of the head. With extension, a cranial force is exerted in the catheter tip region, and with flexion, a force directed caudally. The ligature cranial to the catheter tip can sustain this force initially, but later is not effective and dislocation can occur.

c. In one patient sepsis due to a catheter infection supervened, which necessitated removal of both catheters. After an interval of 2 weeks, the occipital artery was again cannulated and the treatment completed.

d...Catheters occluded by thrombosis did not occur in our patients. It should be noted that most catheters remained in situ for longer than 5 weeks and none had to be removed because of occlusion.

3.2. Systemic Toxicity.

Systemic toxicity of cytostatic agents, if not exceeding certain limits, may not be considered as a complication because side-effects are inherent in the treatment. Minor complications are those side-effects of a cytostatic agent which are (almost) reversible. Serious complications are those side effects which cause irreversible organ function compromise or contribute to the death of the patient. The expected toxicities for Adriamycin and Bleomycin are briefly summarized:

Minor expected toxicities of Adriamycin administration are:

1. Skin and mucosal effects
   a. alopecia
   b. hyperpigmentation at pressure sites
   c. mucositis (see under local toxicity)
2. Gastro-intestinal toxicity
   a. anorexia
   b. diarrhoea
3. Reversible myelosuppression

In our patient study, the myelosuppression was moderate, leucopenia and thrombocytopenia was never greater than Grade 1 (Appendix III). Blood transfusion was necessary in 3 of the 16 patients because the haemoglobin concentration fell to less than 6 mmol/l.

Alopecia (also due to the combined administration with Bleomycin) occurred in all patients. Hair growth resumed in the non-irradiated area in all cases (Grade III, Appendix III).

Severe expected toxicity of Adriamycin administration is:

Myocardial damage, described with total doses exceeding 550 ml/m^2 body surface area (Blum and Carter, 1974), but was not observed in our patients. The maximal total cumulative dose in our study was 210 mg.

Minor expected toxities of Bleomycin administration are:

1. Skin and mucosal effects
   a. alopecia
   b. hyperpigmentation at pressure sites
   c. pressure ulcers (in immobile patients)
   d. mucositis } see under local toxicity
   e. dermatitis }
   f. cheilitis
2. Gastro-intestinal toxicity
   a. anorexia
   b. nausea (mild)
   c. vomiting (mild)
   d. ulceration (duodenal, gastric)
3. General toxicity
   - fever (higher than 40 C (Grade III, Appendix III)), which often occurs during the first administrations and disappears precipitously 4 - 12 hours after completion of infusion (Blum et al., 1973)).

Treatment of minor complications is primarily symptomatic. Alopecia developed in 100% of our patients (also due to the combined administration with Adriamycin) and was reversible in the non-irradiated areas (Grade III, Appendix III).

In one patient, severe gastric haemorrhage occurred, probably from a pre-existent ulcer following Ascal treatment; transfusion was necessary.

Fever (higher than Grade III, Appendix III) occurred in 60% of patients. One patient experienced Grade IV (Appendix III) hyperpyrexia, and it was decided to cease Bleomycin therapy and continue with Adriamycin as monochemotherapy (Case 15).

Severe expected toxicity of Bleomycin therapy.

Pulmonary toxicity is the most serious long-term complication of Bleomycin therapy. Elderly patients receiving cumulative doses of greater than 400 mg Bleomycin are at risk (Blum et al., 1973). The early symptoms are non-productive cough, dyspnoea and fever, and occur 4 -10 weeks after initiation of therapy (Evans et al., 1981). Chest radiography demonstrates

bilateral basilar infiltration which may progress to diffuse interstitial pneumonitis. The treatment is symptomatic.

Some investigators have reported a greater risk of pulmonary toxicity after intravenous pulse administration than after intra-arterial and intravenous continuous infusion (Haas et al., 1976). However, other authors could not find a significant difference in the incidence of pulmonary toxicity due to administration route.

In our patients no irreversible lung lesions attributable to Bleomycin administration were diagnosed. The maximum total cumulative dose for Bleomycin in our study was 180 mg. The most common life-threatening complication during the combination treatment was broncho-pneumonia, which must be treated early. Because fever related to Bleomycin administration can mask a beginning broncho-pneumonia, any increase in temperature after the first week of concurrent combined treatment must be interpreted as a sign of infection and treated as such in order to minimize the risk to the patient's life.

Ten of the 16 patients sustained broncho-pneumonia on one or more occasions, usually after the second week of combined treatment. Two of the 16 patients (Cases 5 and 15) died during the third week of combined treatment, one from toxic shock and the other from extensive broncho-pneumonia.

3.3. Local Toxicity.

Mucosal toxicity is classified in four grades (WHO, 1979). In all our patients, mucositis and cheilitis (Grade III - IV, Appendix III) occurred after two weeks (after half the combined treatment). A Grade III dermatitis always occurred if the skin was in the intra-arterially perfused area (infusion of the facial or transverse facial arteries, Fig. 10.). The skin and adnexa healed with some atrophy (Fig. 11.); mucosal healing in the region of combined treatment with intra-arterial chemotherapy and radiotherapy was always associated with atrophy (Figs 12 and 13). Dermatitis (Grade I - II) was observed if the skin was exposed concurrently to radiotherapy and recirculating cytostatic agents. The treatment of dermatitis is symptomatic.

Massive necrosis of the tumour with debris, mucus, pus and crusts in an oral cavity which is difficult to clean (due to mucositis and pain) predisposes to upper respiratory tract infections which can increase the mortality of treatment. The motivation of the patient and nursing staff is severely pressed by this situation after the second combined treatment week.

Increased local toxicity is found in almost all the

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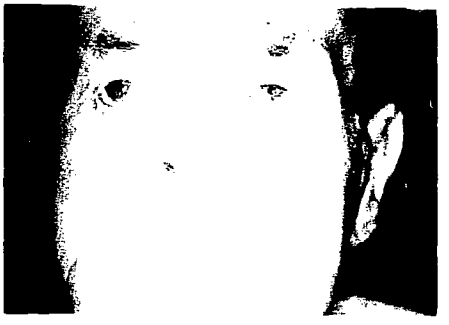


11

12

13

Fig. 10
Pre-auricular Grade III dermatitis after 3 weeks' treatment with intra-arterial Adriamycin and Bleomycin and radiotherapy. In this case the transverse facial, caudal auricular, and the zygomatico-orbital arteries were perfused because ligation was not possible (Case no. 2).

Fig. 11
The patient in Fig. 10, 4 weeks into the recovery period.

Fig. 12 Fig. 13
The patient in Fig. 10, after a recovery period of 4 weeks. The left lingual mucosa shows atrophy which extends over the mid-line at the tip, probably due to retrograde infusion of the tip of the tongue through lingual artery anastomoses. This phenomenon occurred in all cases in which the lingual artery was selectively infused (see also Fig. 13).

literature concerning combined treatment. Esser and Wannenmacher (1979) saw a clear dose-dependent mucositis associated with Bleomycin in concurrent combined treatment with radiotherapy. Sixty-five percent of patients sustained severe mucositis (Grade III and IV) on dose schedules of 2 x 7.5 mg Bleomycin and 500 cGy radiotherapy. With a 2 x 15 mg

Bleomycin and 500 cGy dose schedule severe mucositis (Grades III and IV) developed in 91% of patients treated. Fu et al. (1979a) observed Grades III and IV mucositis in 8 of 15 (53%) patients undergoing combined polychemotherapy and radiotherapy. Curioni and Quadu (1978) saw 100% mucositis (Grades I - IV) in the region perfused by intra-arterial polychemotherapy administered as induction chemotherapy.

In summary, high local toxicity means that concurrent intra-arterial polychemotherapy and radiotherapy is associated with the following disadvantages:
1. Protracted hospitilization period (in part due to the long recovery phase) and associated high cost.
2. Intensive medical and nursing supervision, which requires a trained team
3. The severe local dermatitis and mucositis which develops in the second treatment week causes pain and dysphagia, and thereby reduces the motivation of the patient.
4. Increased chances of development of broncho-pneumonia and its life-threatening consequences, resulting from massive tumour necrosis in the presence of compromise of swallowing function.
5. Atrophy of skin adnexa and mucosa in the region treated by combined therapy.

4. Statistical Correlation between no Evidence of Disease (N.E.D.) and DNA Flow Cytometry, Bone Invasion and Histology.

4.1. Introduction.

Evaluation of the results of treatment involved dividing the patients into 2 groups. In Group I loco-regionally no evidence of tumour after short-term follow up was observed (N.E.D.) (clinically and histologically Groups A and B, Fig. 9). In Group II loco-regional evidence of disease was observed (E.D) (Groups C and D, Fig. 9). One patient could not be allocated to one of these groups (Case No. 6, because she died soon after the completion of therapy with a complete clinical remission and post mortem examination was refused.

The 2 groups were evaluated according to the following parameters:
A. At initial staging (histological grading, extent of bone invasion, DNA index).
B. After cervical dissection (histological evidence of tumour in the dissection specimen).
C. After 2 weeks' combined treatment (analysis of histograms from the treatment period, extent of tumour regression after 2 weeks' treatment).

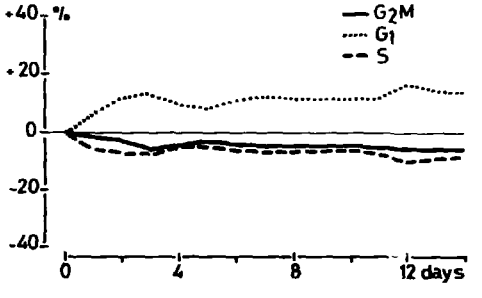
4.2. The Relationship between Parameters Derived From Flow Cytometry and N.E.D.

The treatment-associated changes in the distribution of tumour cells over the cell cycle were expressed as percentual changes in the original values (Figs 14, 15, 16). Time (days) is plotted on the x-axis, and the percentage change of the original value on the y-axis.

A. There was a highly significant* correlation between the extent of accumulation in the G_2+M-phase and N.E.D. (Table 1, Apppendix II).

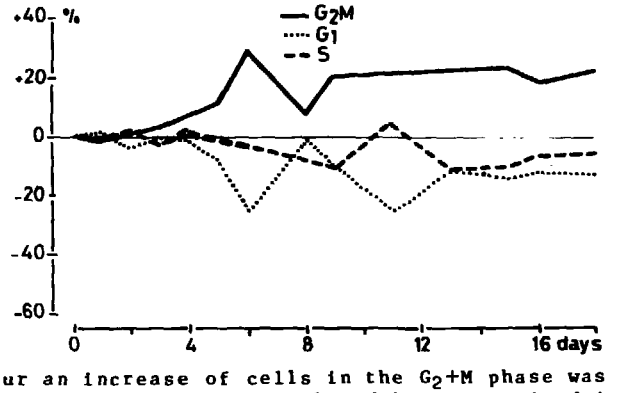
B. No significant correlation was demonstrable between DNA index and N.E.D. (Table 2, Appendix II).

Fig. 14



Therapy-mediated perturbations in the cell cycle distributions of the tumours scored as changes in percentage from control value. In this tumour no increase in the G_2+M phase was evident in any of the biopsies during the concurrent combination therapy. This tumour was scored as 3.: no G_2+M accumulation.

Fig. 15



In this tumour an increase of cells in the G_2+M phase was seen. The maximal effect on day 6 was followed by a sustained increased G_2+M fraction, accompanied by a continuously decreasing G_1 fraction. This tumour was scored as 1.: evident G_2+M accumulation.

Code Table I.

A	Tumour structure	1. solid sheets and/or papillary configuration 2. strands and bands 3. small groups of cells 4. marked cellular dissociation
	Tendency to keratinization	1. highly keratinized 2. some keratinization 3. minimal keratinization 4. no keratinization
	Nuclear aberrations	1. few 2. moderate 3. abundant and few large anaplastic nuclei 4. abundant and many large anaplastic nuclei
	No. of Mitotic figures	1. few (0 - 2) 2. moderate (3 - 4) 3. numerous (5 - 6) 4. extremely numerous (> 6)
	Mode of Invasion	1. well-defined basement membrane 2. less distinct basement membrane 3. no distinct basement membrane 4. no distinct basement membrane and diffuse infiltration
	Inflammatory response	1. marked 2. moderate 3. slight 4. none
A	Classification of bony invasion by X-rays	1. no bone invasion 2. bone invasion 3. extensive bone invasion
A	DNA index	1. not diploid 2. diploid
B	Histological evidence of lymph node metastasis	1. no evidence 2. tumour evidence without capsular invasion 3. tumour evidence with capsular invasion and/or penetration
	Evident G_2+M accumulation after chemotherapy and radiotherapy	1. G_2+M accumulation evident in multiple biopsies 2. G_2+M accumulation evident in one biopsy only 3. no G_2+M accumulation.

TABLE I.

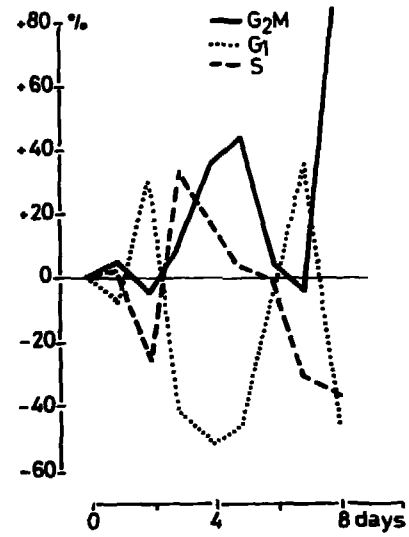
Patient Case No.	Structure	Keratinization	Nuclear aberrations	Mitosis	Mode of Invasion	Inflammatory response	Bone invasion	DNA index	Histological evidence of lymph node metastasis	G_2+M accumulation	Tumour reduction after 2 weeks' concurrent combination therapy	Bone invasion minus nuclear aberrations	2(G_2+M) accumulation + bone invasion
N.E.D.													
2	1	4	3	4	2	3	2	1	3	1		-1	4
5	1	2	3	1	1	2	1	2	3	1		-2	3
7	3	3	2	2	4	4	1	2	3	1		-1	3
8	2	1	2	1	2	2	1	2	1	1		-1	3
12	3	2	3	3	3	4	1	2	1	2		-2	5
13	1	1	3	3	1	2	2	1	3	1		-1	4
16	1	1	2	1	1	3	1	2	3	-		-1	-
E.D.													
1	2	2	2	1	2	4	3	1	-	3		1	9
3	2	2	2	2	3	2	3	2	3	3		1	9
4	2	2	2	2	2	2	1	2	1	3		-1	7
9	3	2	1	1	3	4	3	2	1	3		2	9
10	2	2	2	1	2	2	3	1	3	3		1	9
11	2	2	2	1	3	4	2	2	3	2		0	6
14	4	3	3	1	4	3	3	2	2	2		0	7
15	1	1	2	1	1	2	3	1	1	2		1	7
			A_1				A_2	A_3	B	C_1	C_2		

All patients PR > 50%

(It was not possible to allocate Case No. 6 to either Group)

Table I: The patients from the study were divided into two groups, No Evidence of Disease (N.E.D.) and Evidence of Disease (E.D.) The evaluated parameters are shown; A_1, A_2, A_3 are parameter evaluated at first admission, B are those evaluated post-surgically, and C_1, C_2 are parameters evaluated after two weeks of combined treatment.

Fig. 16



This tumour displayed dramatic cell cycle distributions indicating a synchronization in cell cycle traverse. The huge rise in the G_2+M phase fraction was preceeded by a drop in the S-phase fraction. Changes in the G -phase fraction demonstrate a large growth fraction and/or an extensive and rapid repopulation. This tumour was scored as 1.: evident G_2+M accumulation.

4.3. The Relationship of Histological Evidence of Lymph Node Metastasis and N.E.D.

There was no significant correlation between the presence or absence of histologically confirmed regional lymph node metastasis and N.E.D. Of the 7 patients with N.E.D., 5 had lymph node metastasis in several areas (with capsular infiltration or penetration), and 2 patients did not (Table 3, Appendix II).

4.4. The Relationship between Radiologically Evident Tumour Invasion of Bone and N.E.D.

There was a significant* correlation between the extent of bone invasion and N.E.D. 'No evidence of disease' most often occurred in cases in which the tumour was confined to the soft tissues, with no radiological evidence of bone involvement (Table 4, Appendix II).

---

* tendency to significance $0.05 < P < 0.1$
weakly significant $0.01 < P < 0.05$
significant $0.001 < P < 0.01$
highly significant $P < 0.001$.
(see Appendix II for the P values)

4.5. The Relationship between Histological Grading of the Tumour and N.E.D.

There was no significant correlation between N.E.D. and either tumour structure or the tendency toward keratinization (Tables 5 and 6, Appendix II).

There was a tendency* toward a correlation between the extent of nuclear aberrations and N.E.D. (Table 7, Appendix II).

There was no correlation observed between the number of mitoses and N.E.D. (Table 8, Appendix II).

In grading tumour malignancy by histological classification of the tumour-host relationship, the mode of invasion and extent of inflammatory reaction were both evaluated. Neither showed a correlation with N.E.D. (Tables 9 and 10, Appendix II).

4.6. Multiple Regression Analysis of G_2+M Accumulation and Bone Invasion, with Respect to N.E.D.

Multiple regression analysis of the G_2+M accumulation and bone invasion parameters showed the regression coefficient for G_2 +M to be twice that of bone invasion. There was a highly significant correlation between N.E.D. and the parameters 2(G_2+M) accumulation and bone invasion (Table 11, Appendix II).

2(G_2 +M) + bone invasion as combined parameters give an average of 5.8. The discriminative function is then given by:
2(G_2+M) + bone invasion = 5.8 or:
G_2+M = -0.5 bone invasion + 2.9
A graphic representation of the above discriminative function is shown in Figure 17. Under the discriminative line there is a high probability of N.E.D.

4.7. Multiple Regression Analysis of Bone Invasion and the Extent of Nuclear Aberrations with Respect to N.E.D.

At the start of treatment, when there is no information concerning G_2+M changes, attention must be paid to the extent of bone invasion and the degree of nuclear aberrations (see 4.5.). Analysis showed that there was a highly* significant correlation between these parameters and N.E.D. (Table 12, Appendix II).

---

* tendency to significance $0.05 < P < 0.1$
weakly significant $0.01 < P < 0.05$
significant $0.001 < P < 0.01$
highly significant $P < 0.001$.
(see Appendix II for the P values)

Fig. 17

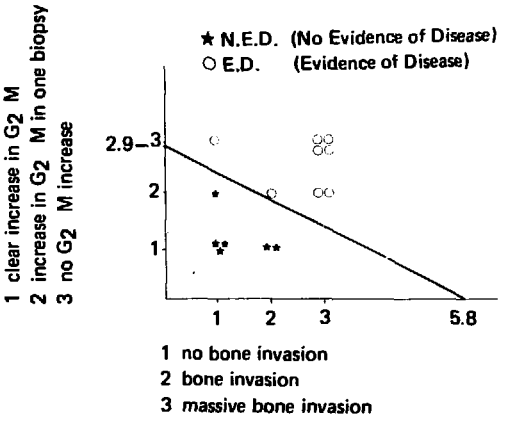


Fig. 18

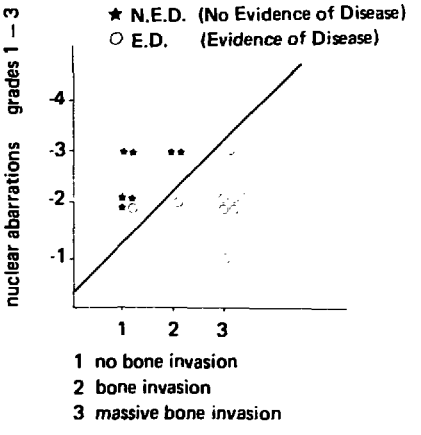


Fig. 17
A graphic representation of the multiple regression analysis of G_2+M accumulation and bone invasion with respect to N.E.D.

Fig. 18
A graphic representation of multiple regression analysis of bone invasion and the extent of nuclear aberrations with respect to N.E.D.

Multiple regression analysis revealed that the discriminative function is given by: Bone Invasion - Nuclear Aberrations = -0.3.
A graphic representation of the above discriminate function is shown in Figure 18. Above the descriminative line there is a high probability of N.E.D.

Multiple regression anaylsis of all the parameters found to be significant (bone invasion, nuclear aberrations, and G_2+M accumulation) showed that nuclear abberations contributed little to the discriminative power of the two other parameters.

PART III

DISCUSSION AND CONCLUSIONS

APPENDIX I,II,III,IV

SUMMARY

SAMENVATTING

CURRICULUM VITAE

DANKWOORD

LITERATURE

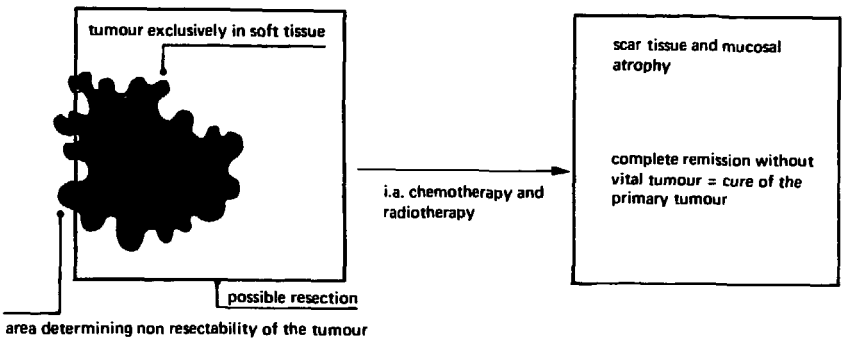
DISCUSSION AND CONCLUSIONS

1. This research began with the supposition that cell cycle perturbations might provide an indication of either the sensitivity of a particular tumour to the concurrent combination treatment used and/or the individual sensitivity to the drugs used. For this reason, investigation of the effect of the drugs on cell cycle distribution was carried out before the actual combination treatment, in order to determine the susceptibility or resistance of the tumour cells to the cytostatic agents used. This provided information concerning the susceptibility of the tumour to Adriamycin, Bleomycin, combination of Adriamycin and radiotherapy, and combination of Bleomycin and radiotherapy. The effects on the cell cycle of Adriamycin and Bleomycin alone were not realized because the chemotherapeutic agents often had no clear effects on cell kinetics. This may be caused by the length of the cell cycle time (Tc) of oral cavity carcinoma which is possibly so long that the first effects of therapy occur relatively late. It is also possible that accumulation in the G_2+M fraction are missed in daily biopsies.

In studies with cell lines Linden et al. (1973) and Reddy et al. (1977) found that G_2+M accumulation of separate therapy models can not be simply added when the therapies are combined. However, others (Schumann and Göhde, 1974; Zywietz and Jung, 1980) did find an increase in synchronization with combined treatment modalities. This still gives no indication of the possible increased effectivity of combination treatment, as it is known that cell death in different phases of the cell cycle actually delays synchronization. The complexity of different phenomena, acting singly or together during treatment (synchronization, cell death, recruitment, etc.), renders it difficult to arrive at an explanation for the presence or absence of changes in cell cycle distribution. The present investigations recorded whether or not cell cycle perturbations actually occurred during the course of therapy.

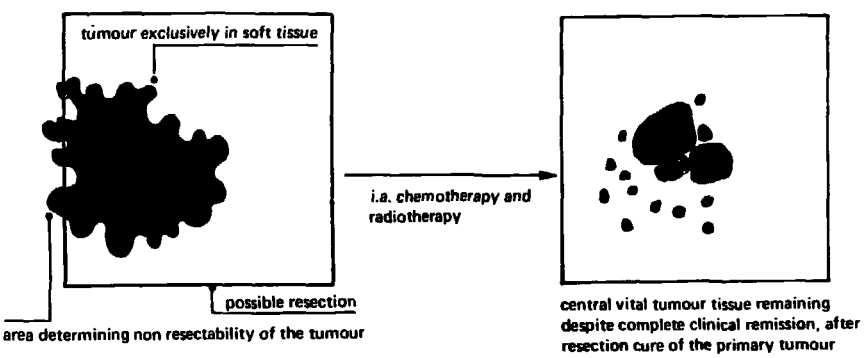
2. Loco-regional sterilization of non-resectable oral cavity carcinoma can be achieved by concurrent intra-arterial chemotherapy and radiotherapy. Clinically complete tumour regression occurred in all cases in which combination therapy was completed. This did not, however, allow prediction of a cure. In two thirds of the cases with clinically complete response (CR) recurrence was seen after a short follow-up (Helpap et al., 1979). It can be said however, that patients with a complete response have a higher survival rate than those with partial response (Snow and Sindram, 1973, Hollmann et al., 1979; Szepesi et al., 1985; Molinari,

Schema I

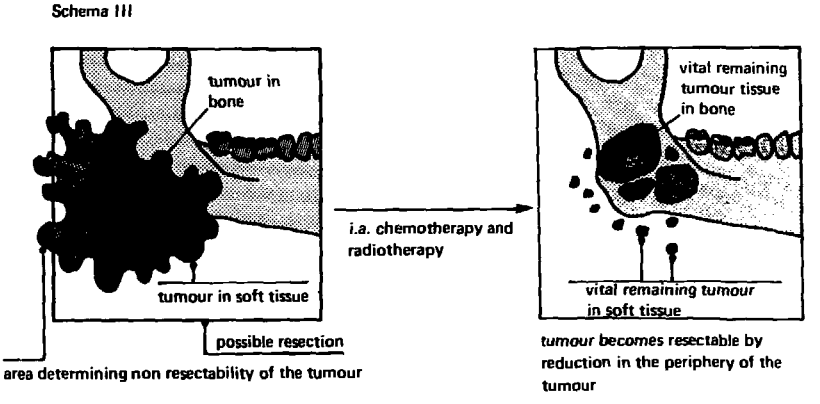


Schema I Oral cavity carcinoma restricted to the soft tissues. Intra-arterial chemotherapy concurrently combined with radiotherapy leads to cure of the primary tumour.

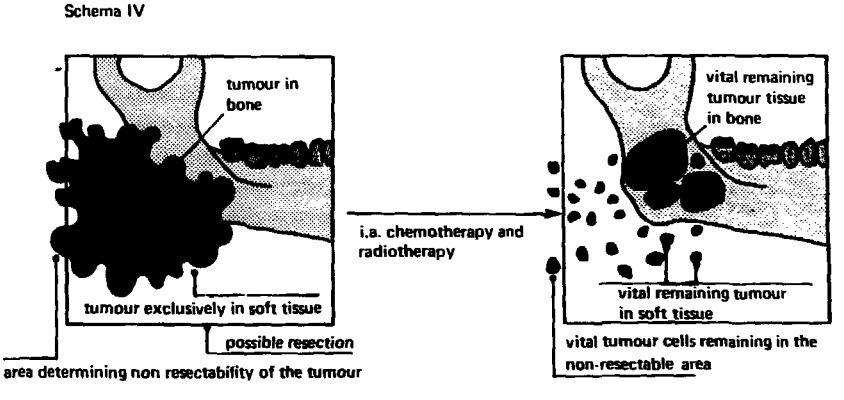
Schema II



Schema II Oral cavity carcinoma restricted to the soft tissues. Intra-arterial chemotherapy concurrently combined with radiotherapy leads to downstaging of the tumour. The tumour is now resectable.



Schema III Oral cavity carcinoma with extension into the bony structures. Intra-arterial chemotherapy concurrently combined with radiotherapy leads to downstaging of the tumour. The remaining vital tumour is resectable.



Schema IV Oral cavity carcinoma with extension into the bony structures. Intra-arterial chemotherapy concurrently combined with radiotherapy leads to clinically complete remission but vital tumour cells remain in the non-resectable area.

1985).

That a high percentage of our patients (6 of 14) developed a local recurrence despite complete response, must be explained by incomplete sterilization of the tumour cells. Regrowth of the tumour can occur from:
a. vital cells in the centre of the tumour (Schemas II and III), in which conditions are less favourable for chemotherapy because of poor perfusion (Tannock, 1968, 1972 and Pavelic et al., 1981).
b. therapy resistant vital cells, which are spread through the whole original tumour area (Schema IV).

In this latter case, the tumour is still non-resectable despite clinically complete response. However, if resection boundaries are tumour-free due to the combination treatment, the tumour is resectable (Schemas II and III). Then the concurrent combined treatment has effected a down-staging of the tumour.

The present study appears to indicate an increased risk in the presence of massive bone invasion by the tumour. Hollmann et al., (1984) and Szepesi et al, (1985) also found a poorer prognosis associated with bone involvement. It is therefore necessary in cases of massive bone involvement, after combined intra-arterial chemotherapy and radiotherapy, to perform resection of all the bony structures involved at initial staging, independent of other clinical, histological and flow cytometric findings (Schema III).

If living tumour cells were found in the edge of the operative specimen removed at resection, an (early) local recurrence occurred despite the multi-modality treatment (Schema IV).

Initially in the present study, subsequent surgery was withheld until tumour recurrence was apparent. If subsequent surgery had been performed after combined therapy in patients with bone invasion and/or an absence of accumulation in the G_2 +M-phase induced by the concurrent combination therapy, the treatment results for the whole patient group would probably have shown higher cure rates. Waiting for recurrence before executing salvage surgery did not result in cures in any of our patients. Patients who could have been surgically cured after combined treatment (Schemas II and III) were probably unable to be cured after manifestation of local recurrence. Additionally, inspection and palpation are often more difficult after combined chemotherapy and radiotherapy, which increases the probability of recognizing recurrences at too late a stage.

In our investigations, there was a highly significant correlation between cell cycle perturbations (G_2 +M accumulation) and "no evidence of disease" after short-term

follow up (Part I, Chapter XI, 4.2.). If no obvious changes in the cell-cycle distribution were seen, there was an increased risk that vital tumour tissue would persist after combination treatment. Wide resection of the original tumour area (except where this is impossible in the non-resectable area) is therefore recommended in cases in which sterilization in the periphery has possibly occurred (Schemas II and III) (successful "down-staging").

Multiple regression analysis of G_2 +M accumulation, bone involvement, and extent of nuclear aberrations demonstrated that the last parameter contributed little to the discriminative power of the other two parameters. Therefore, in planning therapeutic strategy of non-resectable oral cavity carcinoma, histological grading is only of importance at the start of treatment (Ch. X, 2.8., Fig. 8 - Decision Chart). The extent of bone involvement and the analysis of DNA histograms after two weeks of combination treatment provide the most important information concerning further treatment strategy (Fig. 8.).

Any tumour classification system has an inherent weakness because of the many intrinsic tumour-specific features. The TNM classification systems used for head and neck tumours (AJC, 1976; UICC, 1976) are inadequate in producing homogeneous groups to test adjuvant therapies (Harrison, 1978, 1979; Platz et al., 1982, 1983) or combined treatment modalities with drugs and ionizing radiation. In our study, monitoring cell cycle perturbations induced by therapy, was of value, together with other parameters such as histological grading and degree of bone involvement before treatment, in predicting tumour behaviour. For further progress in this field, the classification systems must be refined to allow more individual treatment with radiotherapy and chemotherapy. The nuclear aberrations were of minor importance in this study, but perhaps these data can be of more value after evaluation using a more objective method such as morphometric analysis of nuclear aberrations. Further studies in this area using our patient material are planned.

3. On the basis of data from the literature, Kreidler and Petzels' (1983) method of combined local selective intra-arterial chemotherapy and radiotherapy appears to be the best (Part I, Chapter VIII, Table VII). This protocol was, therefore, largely adopted in the present study.

According to the protocol of Kreidler and Petzel (1983) (Part II, Chapter X, 2.), intra-arterial Bleomycin infusion was performed on two successive days and then followed by radiotherapy. The goal of this regime was to increase the synchronization effect. If the cells are blocked in the G_2 +M-phase for longer than 48 hours, a greater fraction of the proliferating cells are in this (radiotherapy-sensitive) phase (Sinclair and Morton, 1966), because the average cell

cycle time is longer than 24 hours in most squamous cell carcinomas of the head and neck (Part I, Chapter II.).
The above reasoning is plausible on the basis of the synchronization phenomenon, although synchronization alone is responsible for only a small part of a possible supra-additive effect on tumour cells in concurrent combined treatment (Part I, Ch VI, 3.1. Fig. 12).

There are other phenomena which may contribute to the supra-additive effect (Part I, Chapter VI, 3.1., Fig. 12), such as recovery inhibition or fixation of sub-lethal radiation damage by chemotherapy, and inhibition of DNA synthesis by chemotherapy, thereby rendering the cells more sensitive to radiotherapy (Dethlefsen, 1979 a). Such effects are probably better exploited by high intra-nuclear concentrations of Blemomycin, explaining why optimal sequences differ little from the values found by Matsuzawa et al. (1972) using almost simultaneous administration of chemotherapy and radiotherapy.

That synchronization alone can not be responsible for the therapeutic effect on the tumour is evident from the studies of Wannenmacher et al. (1974) and Esser and Wannenmacher (1979), who, despite better synchronization with Bleomycin than with 5Fu, did not observe improved clinical results in the patient group treated with Bleomycin (Part I, Ch. III, 4.).

For Adriamycin the maximal utilization of other phenomena, apart from synchronization, (such as recovery inhibition or fixation of sub-lethal radiation damage and inhibition of tumour cell DNA synthesis rendering the cells more radio-sensitive) can best be obtained by employing as high nuclear concentrations as possible. Excluding synchronization an interval longer than 24 hours after completion of intra-arterial infusion must by far exceed the optimal interval, because the intra-nuclear concentration has again fallen. A protocol in which chemotherapy infusion is performed at every irradiation thus appears more appropriate than the protocol used in the present study. According to Bistrovic et al. (1978) radiotherapy is optimally administered 3 hours following Adriamycin administration.

4. The mortality of combination treatment was high (2 of 16 patients). In the literature, the least toxic combination treatment, namely the sequential administration of radiotherapy with intravenous chemotherapy, was associated with treatment related death rates of 10 and 15% in the studies of Malakar et al. (1980) and O'Connor et al. (1980a,b.) respectively. In more toxic regimes of concurrent radiotherapy and chemotherapy with a multi-drug protocol, Fu et al. (1979a) observed lethal complications in 33% of cases and Fairman (1982) in 36%.

The information derived from the present study, although based on a limited number of patients and a short follow up, allows some conclusions to be made:
Although the protocol used involved some complications in all patients, it is a treatment which can effect cures in non-resectable squamous cell carcinomas of the oral cavity.

DNA flow cytometry on biopsies taken daily during combined treatment showed a clear accumulation of cells in the G_2+M-phase in some of the patients. This accumulation apeared to be highly correlated with 'no evidence of disease' after a short-term follow up in our investigation.

These conclusions give rise to some suggestions for further studies into combined therapy of non-resectable oral cavity carcinoma:

Several combination regimens which include Cisplatinum have been most thoroughly investigated in head and neck cancer, and most involve neo-adjuvant chemotherapy protocols. These resulted in good initial response, but complete response did not exceed 54% (Kish, et al. 1984b.). Small intra-arterial doses of Cisplatinum can be used as a radiosensitizor according to a feasibility study (Molinari, 1985). Concurrent combined treatment modalities with intra-arterial Cisplatinum and single or multiple daily sessions of radiotherapy is in our opinion, the next step in the evaluation of this treatment method.

Well-designed studies in multi-disciplinary clinics which are familiar with intra-arterial catheterization techniques and have facilities for monitoring cell cycle perturbations during therapy, are warranted. It would then be possible to conduct randomized studies involving comparison with already existing trials of treatment with curative intent of non-resectable squamous cell carcinomas of the head and neck.

APPENDIX I

Theoretical pharmacokinetic model for determination of the superior effectiveness of intra-arterial infusion in comparison to intravenous administration. In the theoretical calculation of the superior effectiveness of intra-arterial administration, three assumptions are made. Firstly, the distribution of the drug is described by a two compartment model; secondly, the target area is served exclusively by one artery, without collateral circulation and thirdly, both Target Area Absorption and Absorption-Excretion-Detoxification are supposed to be directly proportional to the local concentration of the chemotherapeutic agent.

The variables used are:

TD = total dose
ΔT = the time during which the cytostatic agent is administered
$\frac{TD}{T}$ = amount of cytostatic agent administered per second
α = flow through the target area
d_i = absorption of the cytostatic agent in the target area
d_s = absorption/excretion/detoxification of the cytostatic agent in the rest of the body
$C_B = C_B(t)$ = amount of cytostatic agent at point B at time t
$C_D = C_D(t)$ = amount of cytostatic agent in the target area at time t
C_B' = amount of cytostatic agent at point B at time $t + dt$
$C_B' - C_B = dC_B$ = change in amount of cytostatic agent after time interval dt
$\frac{dC_B}{dt}$ = change in the amount of cytostatic agent at point B per second.

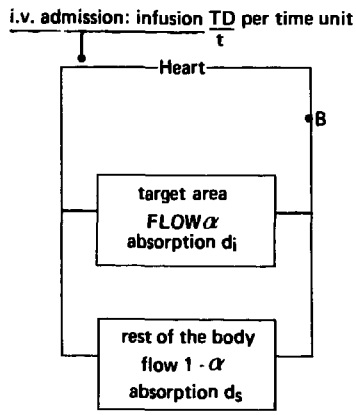
From Equations VA and VB it follows that the amount of the cytostatic agent in the target area after intravenous injection can be expressed as a fraction of the amount of cytostatic agent intra-arterially infused in the target area.
The Rd = the benefit of intra-arterial over the intravenous infusion in the target area during the administration of the cytostatic agent, expressed per unit quantity of the agent

$$R_d = \frac{CD^{i.a.}(t)}{CD^{iv.}(t)} = \frac{VB}{VA}$$

Rd is therefore independant of duration (ΔT) of the infusion and the total dose infused (TD). Both parameters disappear in the ratio of VB and VA.

A: Indication of change in the amount of cytostatic agent in point B during intravenous infusion.

2 compartment model



I A $$C_B + C_B - d_i \alpha\, C_B - d_s\,(1-\alpha)\, C_B + \frac{TD}{\Delta T}$$

II A $$dC_B = \left\{ \frac{TD}{\Delta T} - \left[d_i \alpha + d_s\,(1-\alpha) \right] C_B \right\} dt$$

III A $$\frac{dC_B}{dt} = \frac{TD}{\Delta T} - \left[d_i \alpha + d_s\,(1-\alpha) \right] C_B$$

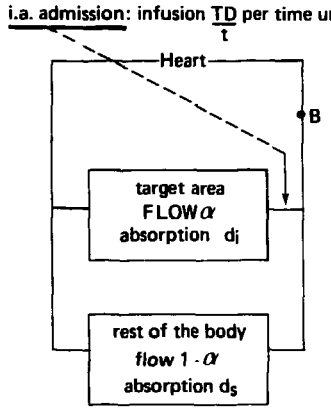
IV A $$C_B\,(t) = \frac{TD/\Delta T}{\alpha d_i + (1-\alpha)\, d_s} \cdot \left[1 - e^{-(\alpha d_i + (1-\alpha) d_s)\, t} \right]$$

Concentration in target area during administration of chemotherapy

V A $$C_D\,(t) = \frac{\alpha\, TD/\Delta T}{\alpha\, d_i + (1-\alpha)\, d_s} \cdot \left[1 - e^{-(\alpha d_i + (1-\alpha) d_s) t} \right]$$

B: Indication of change in the amount of cytostatic agent in point B during intra-arterial infusion.

2 compartment model



I B $$C_B + C_B - d_i\alpha\, C_B - d_s\,(1-\alpha)\,C_B + (1-d_i)\,\frac{TD}{\Delta T}$$

II B $$dC_B = \{(1-d_i)\,\frac{TD}{\Delta T} - [d_i\alpha + d_s\,(1-\alpha)]\,C_B\}\,dt$$

III B $$\frac{dC_B}{dt} = (1-d_i)\,\frac{TD}{\Delta T} - [d_i\alpha + d_s\,(1-\alpha)]\;C_B$$

IV B $$C_B\,(t) = \frac{(1-d_i)\;TD/\Delta T}{\alpha d_i + (1-\alpha)\,d_s}\,.\,[1-e^{-(\alpha d_i + (1-\alpha)d_s)\,t}]$$

Concentration in target area during administration of chemotherapy

V B $$C_D\,(t) = \frac{\alpha\,(1-d_i)\;TD/\Delta T}{\alpha\,d_i + (1-\alpha)\,d_s}\,.\,1 - e^{-(\alpha d_i + (1-\alpha)d_s)t}$$

During i.a. infusion ($t \leq \Delta T$)

$$C_D^{i.a.}(t) = \frac{TD}{\Delta T} + (1-d_i)\, C_D^{i.v.}(t)$$

During recirculation ($t > \Delta T$)

$$C_D^{i.a.}(t) = (1-d_i)\, C_D^{i.v.}(t)$$

Total amount in the target area by i.a. infusion

Total amount i.a. = P + Q

$$P = \int_o^{\Delta T} d_i\, C_D^{i.a.}(t)\, dt$$

$$Q = \int_{\Delta T}^{\infty} d_i \cdot C_D^{i.a.}(\Delta T)\, e^{-k\,(t-\Delta T)} dt$$

in which

$$k = \alpha d_i + (1-\alpha)\, d_s$$

Total amount in the target area by i.v. infusion.

Total amount i.v. = R + S

$$R = \int_o^{\Delta T} d_i\, C_{D.}^{i.v.}(t)\, dt$$

$$S = \int_{\Delta T}^{\infty} d_i\, C_{D.}^{i.v.}(\Delta T).\, e^{-k\,(t-\Delta T)} dt$$

$$R_d^{\infty} = \frac{\int_o^{\Delta T} d_i\, C_D^{i.a.}(t)\, dt + \int_{\Delta T}^{\infty} d_i\, C_D^{i.a.}(\Delta T)\, e^{-k\,(t-\Delta T)}\, dt}{\int_o^{\Delta T} d_i\, C_{D.}^{i.v.}(t)\, dt + \int_{\Delta T}^{\infty} d_i\, C_{D.}^{i.v.}(\Delta T)\, e^{-k\,(t-\Delta T)}\, dt}$$

$$R_d^{\infty} = \frac{d_i \int_o^{\Delta T} \frac{TD}{\Delta T}\, dt + d_i \cdot \int_o^{\Delta T} (1-d_i)\, C_{D.}^{i.v.}(t)\, dt + \int_{\Delta T}^{\infty} d_i (1-d_i)\, C_{D.}^{i.v.}(\Delta T)\, e^{-k(t-\Delta T)} dt}{d_i \int_o^{\Delta T} C_{D.}^{i.v.}(t)\, dt + \int_{\Delta T}^{\infty} d_i C_D^{i.v.}(\Delta T)\, e^{-k\,(t-\Delta T)} dt}$$

$$R_d^{\infty} = \frac{TD}{\int_o^{\Delta T} C_D^{i.v.}(t)\,dt + \int_{\Delta T}^{\infty} C_D^{i.v.}(\Delta T)\, e^{-k(t-\Delta T)} dt} + (1-d_i)$$

$$R_d^{\infty} = (1-d_i) + \frac{TD}{\frac{\alpha D}{k}\Delta T - \frac{1}{k} C_D^{i.v.}(\Delta T) + \frac{1}{k} C_D^{i.v.}(\Delta T)} = (1-d_i) + \frac{k}{\alpha}$$

$$R_d^{\infty} = 1 - d_i + d_i + (\frac{1}{\alpha} - 1)\, d_s = 1 + (\frac{1}{\alpha} - 1)\, d_s$$

$$R_d = 1 + (\frac{1}{\alpha} - 1)\, d_s$$

Conclusions.

A. According to this model, the infusion duration (ΔT) has no effect on the superior effectiveness of intra-arterial infusion, and thus can be chosen by the clinician.
B. The total dose (TD) thus has no effect on the superior effectiveness of intra-arterial infusion (Rd).
C. Equation VIB shows that the concentration in the target area is always higher during intra-arterial infusion than after it, when the concentration is determined only by recirculation (VIIB). During the intra-arterial infusion (VIB) $TD/\Delta T$ can always be computed by the recirculation concentration (VIIB).

The final benefit of intra-arterial administration can be calculated by adding the quantity of cytostatic agent absorbed in the target area before and after the infusion in the case of both intra-arterial (P+Q) and intravenous (R+S) administration, where:
P = the integral of the quantity infused during intra-arterial administration (ΔT)
Q.=.the integral of the quantity recirculated over a sufficient period of time after termination of intra-arterial administration
R.=.the integral of the amount intravenously infused (ΔT)
S.=.the integral of the quantity recirculated over a sufficient period of time after termination of intravenous infusion

$$R_d^{\infty} = \frac{P+Q}{R+S}$$

Further solving of the equation produces $R_d^{\infty} = 1 + (1/\alpha - 1)d_s$ the superior effectiveness of intra-arterial administration. Further conclusions can be drawn:

D. The concentration advantage is dependant to a great degree on the flow of the target area artery (this is equal to the flow at the tip of the catheter,). A decrease in is accompanied by an increase in $\overset{\omega}{R}d$. Chen and Gross (1980) came to the same conclusions through conversion of the equations of Eckman et al. (1974).

E. The superior effectiveness of intra-arterial infusion ($\overset{\omega}{R}d$) is also determined by the absorption - excretion-detoxification by other parts of the body. As ds increases, $\overset{\omega}{R}d$ also increases. The $\overset{\omega}{R}d$ is therefore influenced by the specific properties of the cytostatic agent itself, such as the extent of renal excretion, the extent of hepatic detoxification and excretion, and the binding capacity of the cytostatic agent in other body areas outside the target area.

F. The intra-arterial benefit ($\overset{\omega}{R}d$) is independant of the absorption capacity of the target area (d_i).

APPENDIX II

Table 1

Count / Row Pct / Col Pct	1.00	2.00	Row Total
1.00	5 100.0 83.3		5 35.7
2.00	1 25.0 16.7	3 75.0 37.5	4 28.6
3.00		5 100.0 62.5	5 35.7
Column Total	6 42.9	8 57.1	14 100.0

Table 2

Count / Row Pct / Col Pct	1.00	2.00	Row Total
1.00	2 40.0 28.6	3 60.0 37.5	5 33.3
2.00	5 50.0 71.4	5 50.0 62.5	10 66.7
Column Total	7 46.7	8 53.3	15 100.0

Table 1. G_2+M accumulation versus N.E.D.

Horizontal
1. G_2+M accumulation in multiple biopsies
2. G_2+M accumulation in one biópsy only
3. no G_2+M accumulation

Vertical
1. no evidence of disease (N.E.D.)
2. evidence of disease (E.D.)

The Wilcoxon Test showed a significant* difference with a two-tailed p = 0.0013. The t-test showed a two-tailed p = 0.0001 . There thus appeared to be a significant* correlation between accumulation evident in the G_2+M phase and N.E.D.

Table 2. DNA index versus N.E.D.

Horizontal
1. non diploid
2. diploid

Vertical
1. N.E.D.
2. E.D.

The Wilcoxon Test gave a two-tailed p = 0.7789, and the t-test gave a two-tailed p = 0.738. There is thus no demonstrable correlation between the DNA index and N.E.D.

Table 3

Count / Row Pct / Col Pct	1.00	2.00	Row Total
1.00	2 40.0 28.6	3 60.0 42.9	5 35.7
2.00		1 100.0 14.3	1 7.1
3.00	5 62.5 71.4	3 37.5 42.9	8 57.1
Column Total	7 50.0	7 50.0	14 100.0

Table 4

Count / Row Pct / Col Pct	1.00	2.00	Row Total
1.00	5 83.3 71.4	1 16.7 12.5	6 40.0
2.00	2 66.7 28.6	1 33.3 12.5	3 20.0
3.00		6 100.0 75.0	6 40.0
Column Total	7 46.7	8 53.3	15 100.0

Table 3. Histological evidence of lymph node metastasis versus N.E.D.

Horizontal
1. no evidence of tumour
2. tumour evident in nodes of various regions, without capsular involvement
3. tumour evident in nodes of various regions, with capsular infiltration and penetration

Vertical
1. N.E.D.
2. E.D.

The Wilcoxon Test gave a two-tailed $p = 0.4557$, and the t-test a two-tailed $t = 0.433$. There was thus no demonstrable correlation between the presence or absence of histologically proven regional lymph node metastasis and N.E.D. Of the 7 patients with N.E.D., 5 had proven lymph node metastasis in several areas with capsular infiltration or penetration, and 2 had no lymph node metastases.

Table 4. Extent of bone invasion versus N.E.D.

Horizontal
1. no bone invasion evident
2. bone invasion demonstrable
3. extensive bone invasion

Vertical
1. N.E.D.
2. E.D.

The Wilcoxon Test gave a two-tailed $p = 0.0059$, and the t-test a two-tailed $p = 0.001$. There is thus a significant* correlation between the extent of bone invasion and N.E.D.

Table 5

Count / Row Pct / Col Pct	1.00	2.00	Row Total
1.00	4 80.0 57.1	1 20.0 12.5	5 33.3
2.00	1 16.7 14.3	5 83.3 62.5	6 40.0
3.00	2 66.7 28.6	1 33.3 12.5	3 20.0
4.00		1 100.0 12.5	1 6.7
Column Total	7 46.7	8 53.3	15 100.0

Table 6

Count / Row Pct / Col Pct	1.00	2.00	Row Total
1.00	3 75.0 42.9	1 25.0 12.5	4 26.7
2.00	2 25.0 28.6	6 75.0 75.0	8 53.3
3.00	1 50.0 14.3	1 50.0 12.5	2 13.3
4.00	1 100.0 14.3		1 6.7
Column Total	7 46.7	8 53.3	15 100.0

Table 5. Tumour structure versus N.E.D.

Horizontal
1. solid sheets and/or papillary configuration
2. strands and bands
3. small groups of cells
4. marked cellular dissociation

Vertical
1. N.E.D.
2. E.D.

The Wilcoxon Test gave a two-tailed p = 0.2810 and the t-test a two-tailed p = 0.279. There is thus no significant correlation between tumour structure and N.E.D.

Table 6. Keratinization versus N.E.D.

Horizontal
1. highly keratinized
2. moderate keratinization
3. minimal keratinization
4. no keratinization

Vertical
1. N.E.D.
2. E.D.

The Wilcoxon Test gave a two-tailed p = 0.7789, and the t-test a two-tailed p = 1.00 . There is thus no significant correlation demonstrable between the tendency to keratinization and N.E.D.

Table 7

Count / Row Pct / Col Pct	1.00	2.00	Row Total
1.00		1 100.0 12.5	1 6.7
2.00	3 33.3 42.9	6 66.7 75.0	9 60.0
3.00	4 80.0 57.1	1 20.0 12.5	5 33.3
Column Total	7 46.7	8 53.3	15 100.0

Table 8

Count / Row Pct / Col Pct	1.00	2.00	Row Total
1.00	3 33.3 42.9	6 66.7 75.0	9 60.0
2.00	1 33.3 14.3	2 66.7 25.0	3 20.0
3.00	2 100.0 28.6		2 13.3
4.00	1 100.0 14.3		1 6.7
Column Total	7 46.7	8 53.3	15 100.0

Table 7. Nuclear aberrations versus N.E.D.

Horizontal
1. few
2. moderate
3. abundant, with few large anaplastic nuclei
4. abundant, with many large anaplastic nuclei (not observed in our material)

Vertical
1. N.E.D.
2. E.D.

The Wilcoxon Test gave a two-tailed $p = 0.1206$, and the t-test gave a two-tailed $p = 0.059$. There is thus a tendency* toward a significant correlation between the extent of nuclear aberrations and N.E.D.

Table 8. The number of mitoses versus N.E.D.

Horizontal
1. few (0-2)
2. moderate (3-4)
3. numerous (5-6)
4. abundant (>6)

Vertical
1. N.E.D.
2. E.D.

The Wilcoxon Test gave a two-tailed $p = 0.1893$, and the t-test gave a two-tailed $p = 0.075$ (the t-test appeared to be significant, but remains dubious because of the unequal standard deviation). Therefore, there is no correlation between the number of mitoses and N.E.D.

Table 9

Count / Row Pct / Col Pct	1.00	2.00	Row Total
1.00	3 75.0 42.9	1 25.0 12.5	4 26.7
2.00	2 40.0 28.6	3 60.0 37.5	5 33.3
3.00	1 25.0 14.3	3 75.0 37.5	4 26.7
4.00	1 50.0 14.3	1 50.0 12.5	2 13.3
Column Total	7 46.7	8 53.3	15 100.0

Table 10.

Count / Row Pct / Col Pct	1.00	2.00	Row Total
2.00	3 42.9 42.9	4 57.1 50.0	7 46.7
3.00	2 66.7 28.6	1 33.3 12.5	3 20.0
4.00	2 40.0 28.6	3 60.0 37.5	5 33.3
Column Total	7 46.7	8 53.3	15 100.0

Table 9. Mode of invasive growth versus N.E.D.

Horizontal
1. well-defined basement membrane
2. less distinct basement membrane
3. no distinct basement membrane
4. diffuse invasion

Vertical
1. N.E.D.
2. E.D.

The Wilcoxon Test gave a two-tailed p = 0.3357, and the t-test a two-tailed p = 0.369. There is thus no significant correlation demonstrable between the mode of invasive growth and N.E.D.

Table 10. Extent of inflammatory reaction versus N.E.D.

Horizontal
1. marked inflammation
2. moderate inflammation
3. slight inflammation
4. no inflammation

Vertical
1. N.E.D.
2. E.D.

The Wilcoxon Test gave a two-tailed p = 1.000, and the t-test a two-tailed p = 0.972 . There is thus no demonstrable correlation between the extent of the inflammatory response and N.E.D..

Multiple regression analysis of the parameters G_2 +M accumulation and bone invasion with respect to N.E).

Analysis revealed a regression coefficient for the G_2+M accumulation parameter which was twice as high as that for bone invasion. A weighting factor of 2 was thus chosen for the cross tabulation.

Table 11.

Count / Row Pct / Col Pct	1.00	2.00	Row Total
3.00	3 100.0 50.0		3 21.4
4.00	2 100.0 33.3		2 14.3
5.00	1 100.0 16.7		1 7.1
6.00		1 100.0 12.5	1 7.1
7.00		3 100.0 37.5	3 21.4
9.00		4 100.0 50.0	4 28.6
Column Total	6 42.9	8 57.1	14 100.0

Table 11. 2(G_2+M) accumulation plus bone invasion versus N.E.D.

Horizontal
2(G_2+M) accumulation plus bone invasion
Vertical
1. N.E.D.
2. E.D.
The t-test for 2(G_2+M) plus bone invasion gave a two-tailed p = 0.0001, which is highly* significant.

Multiple regression analysis of the parameters bone invasion and extent of nuclear aberrationd in relation to N.E.D. If there is no information available concerning G_2+M data (in the early stages of treatment), bone invasion and nuclear aberrations must be utilized as parameters (Part II, Ch.XI, 4.5).

Table 12.

Count / Row Pct / Col Pct	1.00	2.00	Row Total
-2.00	2 100.0 28.6		2 13.3
-1.00	5 83.3 71.4	1 16.7 12.5	6 40.0
0.0		2 100.0 25.0	2 13.3
1.00		4 100.0 50.0	4 26.7
2.00		1 100.0 12.5	1 6.7
Column Total	7 46.7	8 53.3	15 100.0

Table 12. Bone invasion minus nuclear aberrations versus N.E.D.

Horizontal
1. bone invasion minus nuclear aberrations
Vertical
1. N.E.D.
2. E.D.
The t-test for bone invasion minus nuclear aberrations gave a two-tailed p = 0.0001, which is highly*significant.

* 0.05 < P < 0.1 tendency to significance
0.01 < P < 0.05 weakly significant
0.001 < P < 0.01 significant
P < 0.001 highly significant

APPENDIX III

W.H.O.: RECOMMENDATIONS FOR GRADING OF ACUTE AND SUBACUTE TOXIC EFFECTS

	GRADE 0	GRADE 1
HAEMATOLOGICAL (ADULTS)	⩾11.0g/100ml	9.5 - 10.9g/100ml
Haemoglobin	⩾110 g/l	95 - 109 g/l
	⩾6.8 mmol/l	5.6 - 6.7 mmol/l
Leukocytes (1000/mm^3)	⩾4.0	3.0 - 3.9
Granulocytes (1000/mm^3)	⩾2.0	1.5 - 1.9
Platelets (1000/mm^3)	⩾100	75 - 99
Haemorrhage	None	Petechiae
GASTROINTESTINAL		
Bilirubin	⩽1.25 x Na	1.26 - 2.5 x Na
Transaminases (SCOT/SGPT)	⩽1.25 x Na	1.26 - 2.5 x Na
Alkaline phosphatase	⩽1.25 x Na	1.26 - 2.5 x Na
Oral	No change	Soreness/erythema
Nausea/vomiting	None	Nausea
Diarrhea	None	Transient, < 2 days
RENAL		
Blood urea nitrogen or Blood urea creatinine	⩽1.25 x Na	1.26 - 2.5 x Na
Proteinuris	No change	1+
		<0.3 g%
		<3 g/l
Haematuria	No change	Microscopic
PULMONARY	No change	Mild symptoms
FEVER WITH DRUG	None	Fever < 38°C
ALLERGIC	No change	Oedema

GRADE 2	GRADE 3	GRADE 4
8.0 - 9.4g/100ml 80 - 94 g/l 4.95 - 5.5 mmol/l	6.5 - 7.9g/100ml 65 - 79 g/l 4.0 - 4.9 mmol/l	<6.5g/100ml <65 g/l <4.0 mmol/l
2.0 - 2.9	1.0 - 1.9	<1.0
1.0 - 1.4	0.5 - 0.9	<0.5
50 - 74	25 - 49	<25
Mild blood loss	Gross blood loss	Debilitating blood loss
2.6 - 5 x Na	5.1 - 10 x Na	>10 x Na
2.6 - 5 x Na	5.1 - 10 x Na	>10 x Na
2.6 - 5 x Na	5.1 - 10 x Na	>10 x Na
Erythema, ulcers; can eat solids	Ulcers; requires liquid diet only	Alimentation not possible
Transient vomiting	Vomiting requiring therapy	Intractable vomiting
Tolerable, but > 2 days	Intolerable, requiring therapy	Haemorrhagic dehydration
2.6 - 5 x Na	5 - 10 x Na	>10 x Na
2 - 3+ 0.3 - 1.0 g/5 3 - 10 g/l	4+ >1.0 g% >10 g/l	Nephrotic syndrome
Gross	Gross + clots	Obstructive uropathy
Exertional dyspnoea	Dyspnoea at rest	Complete bed rest required
Fever 38° - 40°C	Fever > 40°C	Fever with hypotension
Bronchospasm; no parenteral therapy needed	Bronchospasm; parenterial therapy required	Anaphylaxis

CUTANEOUS	No change	Erythema
HAIR	No change	Minimal hair loss
INFECTION	None	Minor infection
CARDIAC		
Rhytm	No change	Sinus tachycardia, >110 at rest
Function	No change	Asymptomatic, but abnormal cardiac sign
Pericarditis	No change	Asymptomatic effusion
NEUROTOXICITY		
State of consiousness	Alert	Transient lethargy
Peripheral	None	Paraesthesias and/or decreased tendon reflexes
Constipation [b]	None	Mild
PAIN [c]	None	Mild

Na = upper limit of normal value of population under study.

b = This does not include constipation resultant from narcotics.

c = Only treatment-related pain is considered, not disease-related pain.

Dry desquamation, vesiculation, pruritus	Moist desquamation, ulceration	Exfoliative dermatitis: necrosis requiring surgical intervention
Moderate, patchy alopecia	Complete alopecia, but reversible	Non-reversible alopecia
Moderate infection	Major infection	Major infection with hypotension
Unifocal PVC, arrhythmia	Multifocal PVC	Ventricular tachycardia
Transient symptomatic dysfunction; no therapy required	Symptomatic atrial dysfunction responsive to therapy	Symptomatic dysfunction non-responsive to therapy
Symptomatic; no tap required	Tamponade; tap required	Tamponade; surgery required
Somnolence < 50% of waking hours	Somnolence > 50% of waking hours	Coma
Severe paraesthesias and/or mild weakness	Intolerable Paraesthesias and/or marked motor loss	Paralysis
Moderate	Abdominal distention	Distention and vomiting
Moderate	Severe	Intractable

APPENDIX IV

CRITERIA OF PERFORMANCE STATUS
KARNOVSKY:

Normal, no complains: no evidence of disease.	100
Able to carry on normal activity. Minor signs or symptoms of disease.	90
Normal activity with effort.	80
Cares for self. Unable to carry on normal activity or to do active work.	70
Requires occasional assistance, but able to care for most of his needs.	60
Requires considerable assistance and frequent medical care.	50
Disabled. Requires special care and assistance.	40
Severely disabled. Hospitalization is indicated though death not imminent.	30
Very sick. Hospitalization necessary. Active supportive treatment necessary.	20
Moribund. Fatal processes progressing rapidly.	10
Dead.	0

SUMMARY

Part I of this thesis is a general section in which various aspects concerning the description of the original research (to be presented in Part II) are outlined.

PART I

In Chapter I the most often utilized staging methods and tumour classification systems are evaluated with respect to their value in providing homogeneous patient populations. The various prognostically relevant factors are discussed. It appears that biological parameters should also be considered in combined chemotherapeutic and/or radiotherapeutic treatment.

In Chapter II the determination, using autoradiography, of cell kinetic parameters of oral cavity carcinoma is presented.

In Chapter III the application of DNA flow cytometry for the biological characterization of oral cavity carcinoma is described. The most relevant determination for prognosis appears to be DNA flow cytometry of tumour biopsies taken during chemotherapy and/or radiotherapy treatment.

In Chapter IV, the influence of chemotherapy and radiotherapy on the progression of the cell cycle is discussed.

Information on the chemotherapeutic agents used is presented in Chapter V.

In Chapter VI the theoretical background of combination treatment with chemotherapy and radiotherapy is discussed. Experimental in vitro and in vivo studies of combined chemotherapy and radiotherapy, with relevance to the present work, are also discussed.

Chapters VII and VIII consist of literature review of the results of intra-arterial chemotherapy and combined chemotherapy and radiotherapy in head and neck squamous cell carcinoma. The various treatment combinations are divided into:
1. phasing of the combined treatment (sequence and interval of administration
2. the different administration routes
3. the different chemotherapeutic agents.

In Chapter IX it is shown that although in clinical studies it is difficult to prove that a superior therapeutic effect can be achieved by intra-arterial administration, there is sufficient evidence to expect a superior effect using this

adminstration route.

PART II

In Chapter X the patient data and combined treatment with local selective intra-arterial chemotherapy and radiotherapy are described. The canulation technique utilized, and the clinical. histological and DNA flow cytometry examination methods used in this series of patients are also described. The chapter concludes with the case histories of the patients treated.

In Chapter XI the results of treatment of the patients previously referred to, are presented, together with the complications and toxicity of simultaneous chemotherpay and radiotherapy.

There is further, an analysis of the clinical, histological and DNA flow cytometry parameters with respect to the patient group which demonstrated 'no evidence of disease' after a short-term follow-up. The statistically significant parameters were determined by means of multiple regression analyses.

PART III

In Chapter XII the results of the previous chapter are discussed and conclusions drawn. The main conclusion is that concurrently combined intra-arterial chemo- and radiotherapy is a treatment which can effect cures in non-resectable squamous cell carcinomas of the oral cavity.

Of the 16 patients in the study, 4 are alive, with no evidence of disease (A - N.E.D.) after short-term follow up. Three patients died, with no evidence of disease (D - N.E.D.).

Thus, in 7 of the 16 patients (44%), no evidence of tumour was observed after short-term follow (A - N.E.D. and D - N.E.D.). These patients were spared death from the effects of the loco-regional tumour process despite the non-resectability of the carcinoma at the time of initial staging.

In spite of the 100% complete clinical remission in 14 patients, there was recurrence in 6 of them. It is thus of importance, that during the treatment the relevant biological parameters are known, so that the prognosis can be determined and the indication for follow-up therapy, considered.

DNA flow cytometry on biopsies taken daily during combined treatment showed accumulation of cells in the G_2+M phase in some of the patients. This accumulation appeared to be

highly correlated with 'no evidence of disease' after a short-term follow up.

It appeared from the study that there is a high chance of local recurrence if massive bone invasion by the tumour is detectable at the initial staging of the tumour. In those cases it is therefore necessary to perform wide resection of the bony structures invaded by the tumour: surgery should follow combined treatment irrespective of other clinical, histopathological or cell kinetic findings.

The sequence and the interval between the used therapy modalities in this study is discussed with respect to the known chemotherapeutic and radiobiological phenomena which contribute to the supra-additive effect of the described combined treatment method.

Finally, some suggestions for further study into combined therapy of non-resectable oral cavity carcinomas are made .

SAMENVATTING

DEEL I van dit proefschrift is een algemeen deel waar in de verschillende actergronden met betrekking tot het beschreven eigen onderzoek (DEEL II) aan de orde komen.

DEEL I:

In Hoofdstuk I worden de meest gebruikte stageringsmethoden en tumorclassificaties getoetst op hun waarde voor het verkrijgen van homogene patientenpopulaties. De diverse prognostisch relevante factoren worden besproken.
Het blijkt noodzakelijk om bij gecombineerde chemotherapie en/of radiotherapie ook biologische parameters in beschouwing te nemen.

In Hoofdstuk II wordt de bepaling van celkinetische parameters van mondholte-carcinomen door middel van autoradiografie beschreven.

In Hoofdstuk III volgt een beschrijving van de toepasbaarheid van DNA flow cytometrie voor biologische karakterisering van mondholtecarcinomen.
DNA flow cytometrie van tumor biopten tijdens behandeling met chemotherapie en/of radiotherapie lijken het meest relevant voor prognostische doeleinden.

In Hoofdstuk IV wordt de invloed van chemotherapie en radiotherapie op de voortgang door de celcyclus besproken.

In Hoofdstuk V volgen enkele gegevens over de gebruikte chemotherapeutica.

In Hoofdstuk VI worden de theoretische achtergronden van de combinatiebehandeling met chemotherapie en radiotherapie besproken.
Eveneens komen experimentele in vitro en in vivo onderzoekingen met de combinatie chemotherapie en radiotherapie, voorzover van belang voor het onderzoek, aan de orde.

In Hoofdstuk VII en VIII wordt een overzicht van de literatuur betreffende de resultaten van intra-arteriele chemotherapie alsmede van de combinatie van chemotherapie en radiotherapie bij hoofd-hals tumoren gegeven.

De verschillende behandelingscombinaties werden opgesplitst in:
1. fasering van de combinatiebehandeling (sequentie en interval van toediening);
2. de verschillende toedieningswegen;
3. de verschillende chemotherapieen.

In Hoofdstuk IX wordt beschreven dat ondanks een groter therapeutisch effect van intra-arteriele chemotherapie in klinische studies moeilijk te bewijzen is, er voldoende aanwijzingen zijn die een superioriteit van de intra-arteriele toedieningsweg aannemelijk maken.

DEEL II:

In Hoofdstuk X wordt het patientenmateriaal en de combinatie behandeling met locaal selectieve intra-arteriele chemotherapie en radiotherapie beschreven. Ook worden de gebruikte canulatietechnieken, de klinische, histologische en DNA flow cytometrische onderzoeksmethoden welke op het patienten materiaal werden toegepast besproken.
In dit hoofdstuk volgen tenslotte de ziektegeschiedenissen van de behandelde patienten.

In Hoofdstuk XI worden de behandelingsresultaten van de voornoemde patientengroep beschreven, alsmede de complicaties en de toxiciteit van de simultane intra-arteriele chemotherapie en radiotherapie.

Verder, volgt dan een analyse van de onderzochte klinische, histologische en DNA flow cytometrische parameters met betrekking tot de patienten groep waarbij loco-regionaal geen tumor werd aangetoond na een korte follow-up.
Door middel van een multipele regressie-analyse worden de statistische significante parameters bepaald.

DEEL III:

In Hoofdstuk XII worden de resultaten uit Hoofdstuk XI besproken en conclusies getrokken. De hoofdconclusie luidt: gecombineerde intra-arteriele chemotherapie en radiotherapie is een behandelings methode die bij niet resectabele carcinomen van de mondholte curatie kan bewerkstelligen.

Van de 16 patienten in het onderzoek, zijn er 4 in leven, zonder aanwijzingen voor tumor na een korte follow-up. Drie patienten overleden aan intercurrente ziekten zonder aanwijzingen (klinische en histologische) voor loco-regionale tumor. In 7 van de 16 patienten (44%) waren dus geen aanwijzingen voor tumor na een korte follow-up. Deze patienten werd een directe dood door het loco-regionale tumor proces bespaard.

Bij 14 van de 16 patienten werd een volledige klinische remissie bereikt. Dit blijkt echter geen garantie voor loco-regionale curatie van de tumor, daar 6 van de 14 patienten een recidief ontwikkelden.

Het is daarom van belang gedurende de behandeling relevante biologische parameters te kennen om zo de prognose te kunnen bepalen en daarmee indicaties voor eventuele vervolgtherapie

te verkrijgen.

Het DNA flow cytometrisch onderzoek aan de hand van dagelijkse biopten tijdens de combinatiebehandeling lieten bij een deel van de patienten een accumulatie van cellen in de G_2 +M fase van de celcyclus zien. Deze accumulatie bleek te correleren met loco-regionale afwezigheid van tumor in de onderzochte patienten groep na een korte follow-up.

Uit het onderzoek blijkt eveneens dat er een verhoogde kans op locaal recidief aanwezig is wanneer massale botingroei door de tumor bij de eerste stagering wordt geconstateerd. In die gevallen is het daarom noodzakelijk om, na de combinatiebehandeling onafhankelijk van de andere bevindingen (klinische-, histopathologische- of celkinetische-), een ruime resectie van de door tumor aangedane bot structuren uit te voeren.

De sequentie en het interval tussen de toegepaste therapie modaliteiten in dit onderzoek wordt besproken aan de hand van bekende chemotherapeutische- en radiobiologische- fenomenen die bijdragen tot een supra-additioneel effect van de beschreven combinatiebehandeling.

Tot slot worden enige voorstellen gedaan voor verder onderzoek met combinatie behandelingen van niet resectabele mondholtecarcinomen.

CURRICULUM VITAE

De schrijver van dit proefschrift werd geboren op 5 juli, 1942 te Bandung (Java).

Hij behaalde in 1964 het eindexamen HBS-B aan de Tymstra HBS te 's-Gravenhage.

In 1965 begon hij zijn studie in de tandheelkunde aan de Rijksuniversiteit te Utrecht. Het doctoraalexamen werd cum laude afgelegd.
Het tandartsexamen werd in 1971 afgelegd. In dat zelfde jaar begon hij zijn opleiding tot specialist in de mondziekten en kaakchirurgie te Utrecht (opleider Prof. J.W.A. Tjebbes). Na diens emeritaat werd de opleiding overgenomen door Prof. dr. P. Egyedi.

Het artsexamen werd in 1976 aan de Rijksuniversiteit te Utrecht afgelegd.

In 1977 werd hij ingeschreven als specialist in de mondziekten en kaakchirurgie.

Van 1977 tot 1979 was hij werkzaam als staflid van de vakgroep mondziekten en kaakchirurgie te Utrecht (hoofd, Prof. dr. P. Egyedi).

Van 1979 tot 1981 was hij werkzaam als Oberarzt op de afdeling Mund- Kiefer- und Gesichtschirurgie (hoofd, Prof. dr. dr. O. Kriens) van het central krankenhaus te Bremen (West-Duitsland).

Van 1981 tot heden is hij wederom also staflid verbonden aan de vakgroep mondziekten en kaakchirurgie te Utrecht.

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