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Perspectives and Commentaries

Of Tumours in Mice and Men, the Different Roles of Somatic Mutation in Treatment Failure*

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In 1979 Goldie and Coldman [1] proposed that the main reason for resistance to cytostatic treatment appearing in recurrent tumours lies in the occurrence in the original cell population of somatic mutations in tumour cells, resulting in the late outgrowth of drug resistant mutant cells. Mouse tumours relapsing during combination chemotherapy are resistant to the cytostatic agents at the concentrations to which they were exposed [2].

At first sight it appears to be similar in human tumours. The finding that non-resistant tumour may recur after stopping of treatment has been explained by either insufficient duration or intensity of the primary treatment (e.g. due to poor penetration of the drug into the tumour), or possibly by reverse mutation among the resistant mutant cells. But in addition another possibility must be considered; the tumour initially grows slowly, but tumour cell progression leads to increasingly faster growth and as a consequence resistance might occur due to fast replacement of drug killed cells.

In how far does this explanation affect the validity of our tumour models such as L1210 leukemia? This long transplanted tumour cell line (and many others) must have undergone in the course of its many passages continuous mutation until a maximal growth rate was reached; most further mutants are likely to grow slower and are therefore rapidly lost from the fast growing population. Yet this effect alone is not enough to explain the absence of resistance to alkylating agents in long transplanted tumour lines. There must be wide differences in the propensity to mutate towards resistance to the alkylating agents and resistance to the majority of antimetabolites and spindle poisons.

Many long transplanted mouse tumours after growth to a palpable tumour of 10^8 cells are curable with cyclophosphamide or one of the nitrosoureas. It seems absurd that on the one hand so many tumours show primary resistance to these agents, whereas other tumours fail to show the expected mutations and remain sensitive to these drugs. This absurdity makes us doubt the postulate that mutation towards cellular resistance to specific drugs is the main cause of treatment failure.

Obviously these long transplanted tumours are different from most early human tumours but similar considerations hold to some extent for human tumours in nude mice. Upon first transplantation and in early passages xenografts shorten their doubling time by a factor of two to six [3]. The slower growing cells that made up the majority of the original population are rapidly overgrown by a few faster growing clones. This implies the likelihood that fast growing cells may be the sole ancestors of all cells remaining after a few passages. If resistant mutants were present in the original population, they were outgrown and lost during these passages. Genetic heterogeneity in the xenograft must mainly originate in the new host.

This makes it very difficult to study the existence of cellular heterogeneity both in the field of drug sensitivity and in proliferation rate in early tumours in patients. We must conclude that it is not only impossible to study the effect of slow proliferation on curability of mouse tumours *in vivo*, but also in all presently available types of human

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tumours. From these considerations it is quite clear that we know much more about the response to cytostatic drugs of the normal host tissue cells, than of the early tumour cells. Drug dosage and scheduling is mostly planned on the basis of avoidance of host toxicity rather than on an assumed improved tumour response.

Nevertheless there is still much heterogeneity in dosage. The excellent review of Hryniuk and Bush [4] has shown for patients treated for relapsed breast cancer a direct relationship between the treatment dose administered per week and the frequency of remission. One aspect of these data is especially striking: the marked increase in frequency of remissions with increase in dose and the apparent lack of influence of these dose increases on the survival time. In this respect it is noteworthy that two papers report on the direct comparison of different schedules of the same drug combination (CMFVP): both Hoogstraten *et al.* [5] and Smalley *et al.* [6] report on the difference between giving a five drug combination dose once every 4 weeks and giving a higher total dose of the same drugs divided in four small weekly fractions. The results, more remissions after the multiple small doses, are closely related to the total dose per 4 weeks given and thus better after the weekly treatment.

This observation indicates that the probability of obtaining a remission is not related to the peak plasma drug level obtained by the treatment but only to the quantity of drug administered per unit time. This implies that failure of a potentially effective combination must depend on two factors: the number of cells killed by the treatment and the time interval between doses. Both studies show a lower effectiveness of an infrequently given high dose, than of a frequently given lower dose per treatment and the paper by Hryniuk and Bush stresses the observation that overall remission frequency in the 18 studies they analysed is directly proportional to the total drug dose per unit time.

It is pertinent here to consider how we must interpret the relation of effectiveness to time in this formula. It seems extremely unlikely that clock time is the relevant factor. The most likely alternative is time in terms of tumour growth. This would imply that the difference in response between different individual tumours could just as well be due to a difference in proliferation rate as to a difference in the fraction of cells killed per unit dose.

In these studies the marked differences in remission frequency do not appear to result in notable differences in patient survival time. It appears that the tumour cells eliminated during the partial or even complete remission are not important in determining terminal disease. There is only one logical explanation: terminal disease is not caused by the tumour cells that are measurable at the time of the first relapse and subsequent partial remission. If our human tumour data indicate a marked increase in tumour growth rate of a small biopsy inoculated into a nude mouse, there is good reason to assume that rapidly proliferating cells may have been present in small quantities in the whole tumour. If the frequency of cells with a double potential growth rate would be around one per million cells, these cell types would be present in one or more of the nude mouse xenografts yet invisible in the human host. Their low quantity at the time of treatment would in most patients not interfere with the appearance of a partial or complete remission and their better survival in the face of chemotherapy would give a logical explanation of the similarity in time of terminal disease and death in treated and untreated patients. We know that in the natural history of primary mouse tumours progression of tumour growth occurs [7] and it would seem likely that the most important type of somatic mutation in human tumours is the continuous progression in growth rate.

For this reason, the more recent papers of Goldie and Coldman [8,9] may be relevant exclusively to transplanted mouse tumours since the authors specifically state that as the basis of their calculation, they assume a constant tumour growth rate.

Many other aspects of treatment are affected by this interpretation. Since in potentially curable tumours resistance to multiple agents has a low frequency at the start of treatment, but rapidly increases during chemotherapy, it would seem logical to precede this chemotherapy by surgical removal of bulky tumour as proposed by DeVita [10]. To this reasoning another point can now be added if we consider that the large tumours are likely to contain cells that are resistant since they grow faster than the treatment can kill them.

What is most disturbing about these modified views into the difference between early primary tumours and transplanted experimental models is their effect on the validity of our tools of study. If a tumour can be drug-sensitive or drug-resistant depending on the cell proliferation rate *in vivo*, we must reconsider why clonogenicity assays can give only incomplete information on the probability of clinical response to chemotherapy. Whereas we originally considered false positive responses as explainable by focal drug resistance missed by our test sample, we must now include the factor of fast repopulation among the reasons for discrepancies. Even more important is the lack of suitable tumour models that can give us information relevant for the phase of slow growth or the stages of growth progression.

The wide differences observed by Hryniak and Bush in the response rates of patients treated with different treatment plans of the same drug combination should make us wary of claims that any test can predict the response to unspecified doses of specified drug combinations. In the cited papers the frequency of partial and complete remissions differed between 66 and 40% [5] and between 46 and 27% [6] depending on the dosage and scheduling differences although each study used the same five drugs in both arms of its controlled clinical trial. This implies a poor predictability of response to this type of combination therapy by any preclinical test.

For the study of adjuvant chemotherapy more attention should be given to animal models of primary tumours (11) even if these models have other disadvantages.

It is also clear that we need more human data to expand the findings of Hryniuk and Bush to other tumour types, preferably including human tumours of which a fraction can be cured with chemotherapy or multimodality treatment.

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