A Rationale for Synchrony Strategies in Chemotherapy

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Introduction

A synchrony strategy in the treatment of a tumor consists of two parts.
First is the introduction of an agent which blocks the tumor cells at some
point in the cell cycle. Second is the release of this block until the par-
tially synchronized tumor cell population arrives in a wave at a vulnerable
part of the cell cycle where it is treated with a phase specific agent.

The effectiveness of a therapeutic regime against cancer is determined
by the amount of damage produced in the neoplastic tissues versus the amount
of damage produced in the limiting normal tissues. The limiting normal tis-
sues are those which first express the damage done by the regime and cause
a limitation of the treatment. The treatment-limiting cell populations in
much of chemotherapy are those with a short cycle time (roughly twenty-four
hours) in the bone marrow and the gastro-intestinal tract.

The basic reason for using a synchrony strategy is that it produces a
greater kill of neoplastic cells compared to treatment-limiting normal cells
than does chemotherapy which does not take account of cellular kinetics.
This therapeutic gain is due to the greater average cell cycle time of the
malignant cells and to random variation in cell cycle times.

We take the synchrony strategy described by Barranco et al. (1) which
was used for the treatment of melanoma as a prototype. Sherakawa et al. (4)

A RATIONALE FOR SYNCHRONY STRATEGIES IN CHEMOTHERAPY

estimate that the cycle time of melanoma is 74 hours, with the mean time in the G_1, S and G_2 phases being 48 hours, 21 hours, and 5 hours respectively. The synchrony strategy employed was to infuse bleomycin for four days (slightly longer than the mean cycle time of the melanoma cells) thus blocking cycling melanoma as well as normal cells at the S/G_2 junction. At the end of this four day period, infusion is ended and the bleomycin is rapidly eliminated from the body. The melanoma nodules are biopsied periodically in order to identify the time when the partially synchronized neoplastic cells enter S-phase. This should be the time required to traverse G_2, M, and G_1, or about two days. Peaks in labeling index were indeed found between two and three days, (1). These peak labeling indices were up to four times the pre-infusion value, although typically they were only twice the pre-treatment value. When the peak occurs, an S-phase specific agent such as hydroxy urea or Ara-C is given. Twice as many malignant cells were vulnerable as would have been had the synchrony treatment not been employed.

In the same two to three days that the malignant cells are progressing through the cycle to S-phase, the normal cells will have traversed their cell cycle two to three times. Due to random fluctuations in the cell cycle time, these cells will have completely desynchronized. Hence, the killing of these cells should be no more extreme than it would be had the synchrony procedure not been employed.

The therapeutic gain obtained by using synchrony over the phase specific agent without synchrony is of the order of two to one for a single treatment. The gain from several treatments should multiply so that if we had ten such treatments we would expect a gain of about one thousand to one and with twenty treatments, about one million to one.

This analysis ignores the fact that most of the tumor cells are not

cycling. In melanoma, the estimates of the growth fraction of the tumor
seem to center around twenty percent (1,4). In the following sections of
this paper, we present a simple model of the changes in growth fraction in
the tumor with treatment and the consequences of these changes to the pos-
sible outcome of a series of treatment utilizing a synchrony strategy.

The model

In Figure 1 we exhibit a simple model of the cycle of a mammalian cell.
There is abundant evidence (2,4,5) that as the tumor increases in size the
growth fraction, i.e., fraction of cells traversing the cell cycle, decreases.
One model discussed by Gilbert and Lajtha (3) proposes that cells which leave
the cycle do so after mitosis and enter the cycle only by returning to G_1.
We refer to the non-cycling cells as being in the G_0 phase.

The quantitative nature of the changes in growth fraction as a tumor
either grows or recedes under treatment seems not to be completely known.
Neither are the mechanisms which cause these changes. However, work by Tan-
nock (5) suggests that the vascularization of the tumor may be an important
factor and we base the following considerations on this concept.

We assume that vascularization of the tumor is sufficient to support a
particular number of cells in cycle and that this is the number of cells
which are cycling prior to treatment. We also assume that the number of cells
which can be in cycle does not change appreciably during the course of treat-
ment.

The cells which are not cycling are not participating in the biochemical
events necessary for reproduction and consequently are not subject to the
disruption of these events by phase specific chemotherapeutic agents. Con-
sequently, these cells are not damaged by the phase specific agents employed.

A RATIONALE FOR SYNCHRONY STRATEGIES IN CHEMOTHERAPY

There are three parameters in our model. The first is the pre-treatment growth fraction. This is held at 0.2 throughout. The second parameter is the proportion of cycling cells killed by each treatment. This parameter is denoted by k. Its value is systematically varied between 0.1 and 0.9 in the following. However, in the cited case of melanoma, S-phase constituted around .3 of the cycle time and synchrony strategies somewhat more than doubled the pre-treatment labeling index, so a k of .7 would seem appropriate. The third parameter, t, is the proportion of additional cells which could be supported in cycle (due to killing of cycling cells by previous treatments) which do in fact transfer from G_0 to the cycling state between treatments. An example may make the meaning of this parameter clearer. Suppose that the vascularization is sufficient to support 1000 cells in cycle. Suppose that a treatment kills 700 of these cells. Then 700 additional cells could be supported in cycle, but if t is 0.9, then only $0.9 \times 700 = 630$ will transfer from G_0 into the cycle before the next treatment. Chart 3 of (1) suggests that t is quite high, probably even higher than the natural 1.0 limit suggested by our model. This chart represents data on only one patient, and we take here the more conservative position that t is 0.9

Using these parameters, the outcome of a course of treatment is easily calculated. For each treatment, the number of cycling cells is reduced to $1.0-k$ of its pre-treatment value. The number of cells which will transfer from G_0 to the cycling state is computed using t as described above. This sequence of calculations is repeated for each treatment.

Results

We look first at the effect of each of our parameters individually. The pre-treatment growth fraction is extremely important in determining the out-

come of a course of treatments. If each treatment produces a kill, $\underline{k}$ of 0.7, and if the transfer parameter is 0.9, then twenty treatments will produce the following surviving fractions of tumor cells: if the initial growth fraction is 0.1, then the surviving fraction is 6.2×10^{-5}; if the initial growth fraction is 0.2 then the surviving fraction is 1.2×10^{-8}; if the initial growth fraction is 0.3, then the surviving fraction is reduced to 8.8×10^{-10}. By our assumptions, a tumor with a growth fraction of 0.1 is about three times the size of a tumor with a growth fraction of 0.3, but this threefold difference in size make a difference of 10^5 in surviving fraction after a course of twenty treatments.

Figure 2 presents the surviving fraction following a course of twenty treatments as a function of the effectiveness, $\underline{k}$, of each treatment. Even in the most optimistic case presented, $\underline{t}$ of 0.9, we require that $\underline{k}$ be at least 0.6 before we get a therapeutically useful surviving fraction of 10^{-6}. Killing rates of 0.3 or less would have virtually no effect on the tumor.

This points out the importance of a synchrony strategy. If phase specific drugs were used without synchrony, they would have to be applied quite frequently to achieve therapeutic effectiveness. This frequent application would do great damage to the rapidly cycling limiting normal tissues. By using a synchrony strategy in the case considered, we should be able to achieve a surviving fraction in the tumor of close to 10^{-8}. If normal tissues have an S-phase which constitutes .3 of the total cycle, the surviving fraction after twenty treatments would be about 10^{-2}. Synchrony has thus given a therapeutic advantage of 10^6.

Chart 3 illustrates the effect of the transfer parameter, $\underline{t}$, on the surviving fraction following a regime of twenty treatments. For low values of $\underline{t}$ the regime has little effect on the tumor and there is little change in

42

A RATIONALE FOR SYNCHRONY STRATEGIES IN CHEMOTHERAPY

surviving fraction with t. For t slightly higher than 0.2, there is a distinct increase in treatment effectiveness with an encrease in t, although there is a leveling off of this change with still higher values of t. It is difficult to make any inferences of use in therapy from this data although the value of t will undoubtably be influenced by the period between treatments.

Figure 4 presents the surviving fraction following each treatment for k equals 0.7 and the same five values of t as figure 1. In each case, there is a shoulder where each treatment causes little incremental killing. Following the shoulder there is a rapid decrease in surviving fraction with each successive treatment. Figure 5 makes the reason for this shoulder effect clear: it presents the growth fraction following each treatment. A comparison of the two figures shows that the shoulder lasts until all of the cells are cycling. Before that point, many of the tumor cells are protected by being in the G_0 state.

It should be noted that chart 3 of (1) indicates that in the one experience in which several synchrony treatments were given to a melanoma patient   ,
it seems that the growth fraction of thé tumor rose to quite close to unity after only two treatments, thus the considerations here may be quite conservative.

The following formula may be used to give a comparison on the length of the shoulder using phase specific drugs with and without synchrony:

Shoulder length (without synchrony) = Shoulder length (with synchrony) X Mean total tumor cycle time / Mean time in sensitive phase.

Thus in our case with k equals .7 and t equals .9, we see from charts 4 and 5 that the shoulder has a length of seven treatments. Without synchrony, according to the formula, the shoulder would be about three times as long or twenty-one treatments. Should the observation in (1) be true that the observed

43

shoulder with synchrony is only two treatments, without synchrony we would
expect a shoulder of six treatments.

Chart 6 presents the therapeutic ratio obtained by the use of synchrony
as a function of the number of treatments in the regime. The three lines
were all computed assuming that each treatment killed 0.7 of the tumor cells
and 0.3 of the limiting normal cells. The top line was computed under the
(optimistic) assumption that all tumor and normal cells are cycling through-
out. The middle line was computed assuming that both the tumor and the limi-
ting normal cells behave according to our model. It does seem that bone
marrow cells have kinetic behavior that protects from destruction in a way
analogous to that discussed for tumor cells. We see that the therapeutic
ratio can still rise to 10^6 in twenty treatments. The shoulder effect is
still noticeable -- the therapeutic ratio is small for the first few treat-
ments and rises rapidly thereafter. Should the shoulder in fact be smaller
than predicted by the model, the therapeutic ratio would be even higher than
given by this curve. The bottom curve was calculated under the (pessimistic)
assumption that the tumor behaved addording to our model with t of 0.9, but
that the limiting normal cells were all cycling throughout treatment. From
the graph we see that the first seven treatments yield a therapeutic ratio
of less than one -- i.e., more damage is done the normal cells than the tumor
cells. The ratio does rise after that, however, and after twenty treatments
reaches 3×10^4.

Conclusions

Several implications for chemotherapeutic strategies can be drawn:
(1) Induced synchrony seems almost a necessity if a large reduction in tumor
size is to be effected by a reasonable number of treatments with phase speci-
fic agents without unacceptable damage to normal tissues. This is emphatically

A RATIONALE FOR SYNCHRONY STRATEGIES IN CHEMOTHERAPY

so if the tumor has a large mass of non-cycling cells.

(2) A course of treatment is more effective on a small tumor in which most cells are cycling than on a large tumor in which most cells are in G_0. It is important to treat early.

(3) A synchrony strategy would be expected to be effective on disseminated disease with distant metastases provided that the kinetics in the metases are similar to each other and to those of the primary.

(4) Initial treatments are relatively ineffective in reducing the overall surviving fraction in the tumor compared to later treatments. It is important .to have enough treatments.

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(5) Tannock, I.F. (1968) The relation between cell proliferation and the vascular system in a transplanted mouse mammary tumor. The British Journal of Cancer XXII:258.
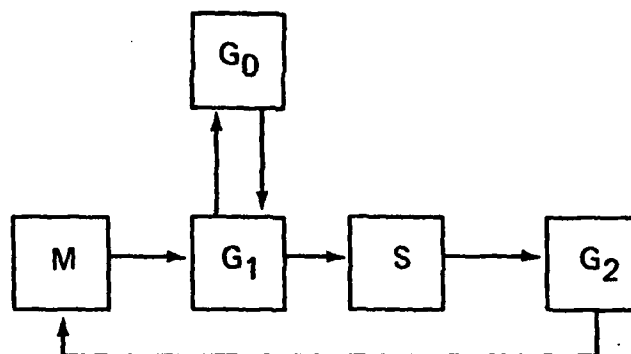
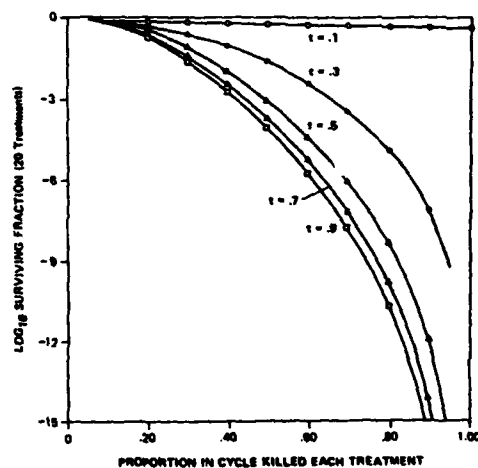Figure 1. A model of the cycle of mammalian cells.

Figure 2. Logarithm to the base ten of the surviving fraction of tumor cells following a regime of twenty treatments, each of which kills a fraction of cycling cells given on the abscissa. The parameter t is the proportion of cells transferred into cycle between treatments. In the case of melanoma, t appears to be at least 0.9. For this case, note the difference in surviving fraction with synchrony where the kill is about 0.7 versus no synchrony where the kill is about 0.3.
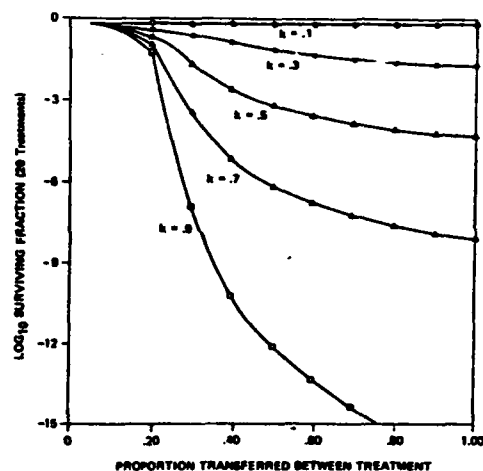
46

Figure 3. Logarithm to the base ten of the surviving fraction of cells following a regime of twenty treatments, each of which kills a proportion of the cycling cells given by k. The abscissa is the proportion of non-cycling cells which could be supported in cycle which do become cycling between treatments.
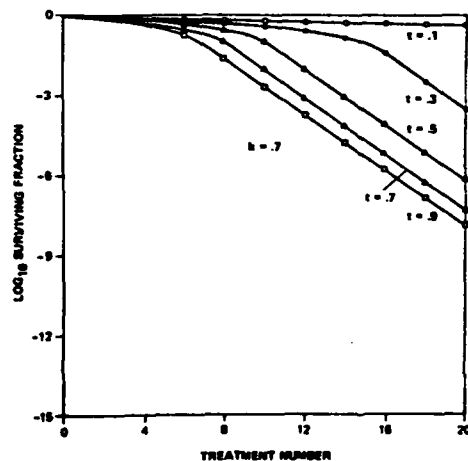

Figure 4. Logarithm to the base ten of the surviving fraction versus the number of treatments. Each treatment kills 0.7 of the cycling cells. The lines represent differing proportions of cells transferring into cycle between treatments. In the case of melanoma, t seems to be at least 0.9. Note the shoulder effect: initial treatments have little effect whereas each subsequent treatment has a pronounced effect.
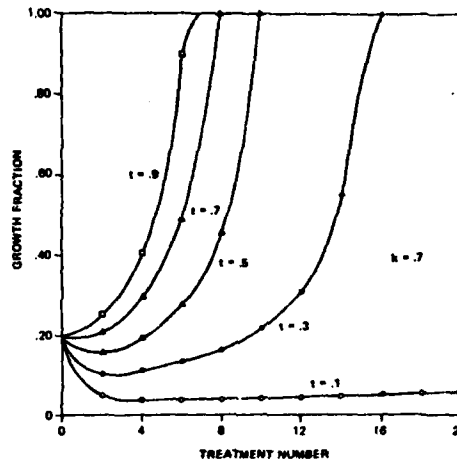
47

Figure 5. Growth fraction versus the number of treatments. The reason for the comparative lack of effectiveness of the initial treatments is that most cells are protected by being in a non-cycling state.
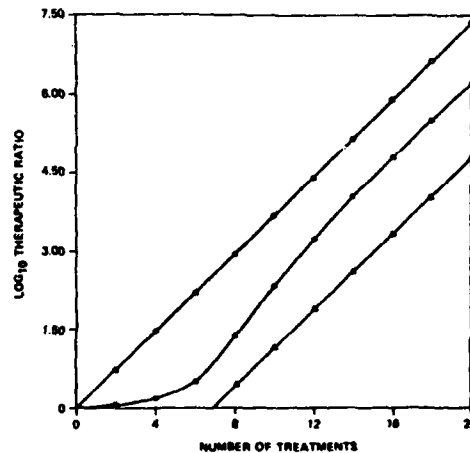

Figure 6. Logarithm to the base ten of the therapeutic ratio versus the number of treatments. The therapeutic ratio is the ratio of surviving fraction of treatment limiting normal tissue to that of tumor tissue. It is assumed that each synchrony treatment kills 0.7 of cycling tumor cells and 0.3 of cycling normal cells and that the parameter $\underline{t}$ is 0.9 for both. The top curve (open circles) is calculated assuming that both tumor and normal tissues are cycling. The middle curve (closed circles) is calculated assuming that both types of tissue behave according to the model. The bottom curve (triangles) assumes that the tumor behaves according to the model whereas the normal cells are cycling. In the last case, seven treatments are required before there is any therapeutic gain but successive treatments greatly increase the gain.

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