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Research Article

Drug Resistance, An Enemy of Targeted Cancer Therapies

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1. Abstract

The success of targeted cancer therapies will depend on the uniformity of the target cells. Cancer cells mutate and potentially can alter that target, defeating that therapy. A means of computing the number of altered targets and what may be necessary to improve the probability of successful treatment is presented. This analysis indicates that single drug, targeted therapy will not be successful unless the cancer can be treated in very early stages with low cancer cell counts.

2. Key words

Cancer; Targeted Therapy; Treatment Failure

3. Practitioner Points

1. The size of a tumor only gives a rough estimate of whether targeted cancer treatment will work
2. The number of cells in a tumor gives a more accurate estimate whether single or multiple targeted therapies will be required for successful treatment.

4. Introduction

More than half a century ago, Edward Tatum made an important observation concerning the evolution of drug-resistance. He wrote about this in his 1958 Nobel Laureate Lecture:

"In microbiology the roles of mutation and selection in evolution are coming to be better understood through the use of bacterial cultures of mutant strains... The therapeutic use of massive doses of antibiotics to reduce the numbers of bacteria which by mutation could develop resistance, is a direct consequence of the application of genetic concepts. Similarly, so is the increasing use of combined antibiotic therapy, resistance to both of which would require the simultaneous mutation of two independent characters."

He went on further to say:

"As an important example of the application of these same concepts of microbial genetics to mammalian cells, we may cite the probable mutational origin of resistance to chemotherapeutic agents in leukemic cells, and the increasing and effective simultaneous use of two or more chemotherapeutic agents in the treatment of this disease [1]."

What Edward Tatum wrote describes the fundamental principles for treating diseases caused by evolving populations. The goal in treating infectious diseases and cancers is to drive those populations causing the diseases to extinction. The greater the selection pressure put on these diseases causing populations, the greater the chance of driving these populations to extinction. This paper addresses an analytic approach for determining the number of selection pressures needed in order to treat a cancer and have a high probability of success.

Cancer cells are not exact clones of the founder cell. There is great diversity in cancer cell lines and unless the targeted cancer therapeutic agent can cross-react with all the possible variants that might exist in a cancer cell line, one should expect treatment failure. The first step in making this determination is to estimate the number of cells in the cancer. This is typically not done when staging cancer. Cancer staging typically determines the location and extent of the cancer but not an estimate of the actual number of cells. "Staging helps describe where a cancer is located, if or where it has spread, and whether it is affecting other parts of the body [2]." The number of cells that can occur in a tumor has been estimated. "The number of cancer cells is a function of tumour volume in cubic centimetres. Each cell is about 20 μm in diameter. A 1-cm cancer has about 100 million cells, a 0.5-cm cancer has about 10 million cells, and a 1-mm cancer has about 100 thousand cells [3]." The mutation rate of cancers has also been estimated. "The mutation rates of cancer cells to drug and multidrug resistance are paradoxically high, i.e., 10^{-3} to 10^{-6}, compared with those altering phenotypes of recessive genes in normal diploid cells of about 10^{-12} [4]." Once the number of cells in the tumor is estimated (and the ploidy), and the mutation rate is estimated, the probability of resistant variants to one or more selection pressures can be estimated.

The estimation of resistant variants in a tumor requires more analysis than the typical staging process. Determination of the location of a cancer and where the cancer has spread is not sufficient. The size of the tumor must be accurately estimated. Typically, staging

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is not this precise. "T1, T2, T3, T4: Refers to the size and/or extent of the main tumor. The higher the number after the T, the larger the tumor or the more it has grown into nearby tissues. T's may be further divided to provide more detail, such as T3a and T3b [5]." Likewise, not only must the size of the primary tumor must be accurately estimated, the number of cancer cells in any metastasis and in lymph nodes must be accurately estimated in order to give a good estimate of the number of resistant variants that exist in that population.

5. Materials & Methods

This analysis is based on the mathematical equations derived in references [6, 7]. The mathematics used and the complete derivation of the equations used to derive the figures which describe the probabilities of resistant variants in a cancer cell population is shown in these two references. These equations were derived to explain the evolution of anti-microbial drug-resistance for the treatment of infectious diseases but with slight modification, these equations are applicable to the evolution of cancer treatment targeted therapy. Drug resistance in both infectious disease treatment and cancer treatment can fail due to the process of random mutation and natural selection. In order for a bacterial population or cancer cell population to evolve resistance, it must do so by accumulating resistance (beneficial) mutations. This process consists of a cycle of beneficial mutation followed by amplification of that mutation (the increase in the number of those members with the beneficial mutation in order to improve the probability of the next beneficial mutation occurring. In order to better understand this process, a simple analogy can be made.

A simple analogy of random mutation and natural selection can be made by imagining that your family must win two lotteries in order to survive. The probability of winning each lottery is 1 in a million. For you to win both lotteries, that probability is 1 in a million times 1 in a million, 1 in a trillion! A very low probability indeed. But let's say that you are lucky enough to win one of the lotteries. You have won one of the lotteries and you are now very wealthy. This wealth allows you to raise a very large family. Now, all your descendants start buying tickets to the second lottery. When you have enough descendants, you have a reasonable probability that one of these descendants will win the second lottery for your family.

We can then apply this principle to random mutation and natural selection. Assume a population requires mutations A, B, and C, to evolve resistance to a drug. Some lucky member of that population gets mutation A. That member must amplify (increase in number) to improve the probability of mutation B occurring on one of its members that already has mutation A. When some lucky member with mutation A gets mutation B, a new sub-population is started of variants which have both mutations A and B. That member with mutations A and B must again amplify to improve the probability of mutation C occurring on one of its members. The reason why amplification must occur between each evolutionary step is to improve the joint probability (the probability of the two beneficial mutations events occurring in a single lineage). When Edward Tatum says, "The therapeutic use of massive doses of antibiotics to reduce the numbers of bacteria which by mutation could develop resistance, is a direct consequence of the application of genetic concepts.", is that if you inhibit A from amplifying, you will have a low probability of B mutation occurring on a member with mutation A.

If one considers the situation where two selection pressures are applied to a population. Let one drug require mutations A, B, and C, and the other drug requires mutations X, Y, and Z for adaptation. Even is some lucky member gets a beneficial mutation, A for the first drug, the second drug interferes with the amplification of that mutation. And if some lucky member gets a beneficial mutation X, for the second drug, the first drug interferes with the amplification of that mutation. These principles can be applied to cancer treatments to determine the number of selection pressures necessary to have a reasonable probability of successful treatment.

The fundamental governing mathematical equation for the probability that a particular mutation will occur was derived in reference [6] and is:

$$P(X) = (1 - (1 - P(\text{Beneficial})\mu)^N)$$

where X is the particular beneficial mutation, P(Beneficial) is the probability of all the possible mutations which could occur at the given site that the beneficial mutation occurs, μ is the mutation rate, and N is the total number of replications of the particular variant that would benefit from mutation X occurring on one of its members. The derivation done in reference [6] was based on the assumption that the replicator was haploid so that each replication of a member was equal to a replication of the genome. However, cancer cells are diploid (or possibly even polyploid). The implication of this is that every cell replication in a cancer represents at least two genome replications and therefore, a population of 100,000 cancer cells would have 200,000 genome replications and N=200,000 in this probability calculation. The probability equations for two and three simultaneous selection pressures are derived and plotted in reference [7] and displayed in the results section. These equations apply to every site in the genome so that if a beneficial mutation can occur at any site, this math applies.

6. Results

The following figures are from reference [6, 7] and are used to calculate the probability of a particular variant occurring as a function of the number of replications of that site (genome) and a given beneficial mutation rate (P(Beneficial)μ). Note that if we assume the cancer is diploid, the number of replications (N) of the DNA will

be twice the number of cells. If the cancer cells are polyploid, the number of replications will be the number of cells times the ploidy of the cells. The probability curves as a function of the number of replications of the genome and mutation rate as a parameter are displayed below (Figure 1).

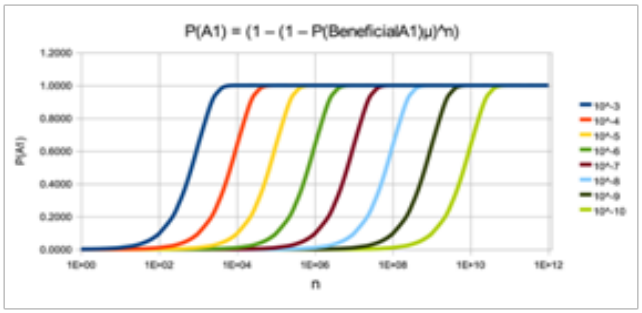

Figure 1: Probability curves for a particular mutation occurring as a function of n, the number of replications for various values of P(Beneficial)μ.

Figure 1 gives the probability of a particular mutation occurring as a function of the number of replications of the particular genome with the beneficial mutation rate as a parameter. These curves give the probability that a cancer cell variant will exist in the tumor cell population with a beneficial mutation to single targeted therapy (Figure 2).

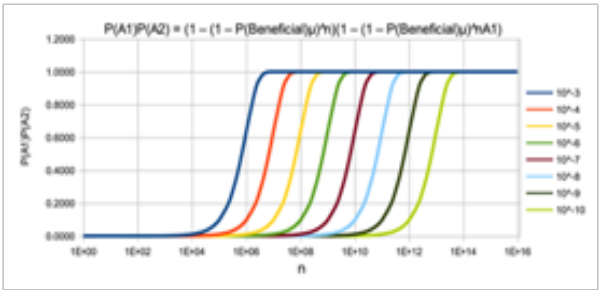

Figure 2: Probability curves for two mutations occurring as a function of n, the number of replications for various values of P(Beneficial)μ.

Figure 2 gives the probability of two particular mutations occurring as a function of the number of replications of the particular genome again with the beneficial mutation rate as a parameter. These curves give the probability that a cancer cell variant will exist in the tumor cell population with beneficial mutations to two targeted therapy or two beneficial mutations to a single targeted therapy (Figure 3).

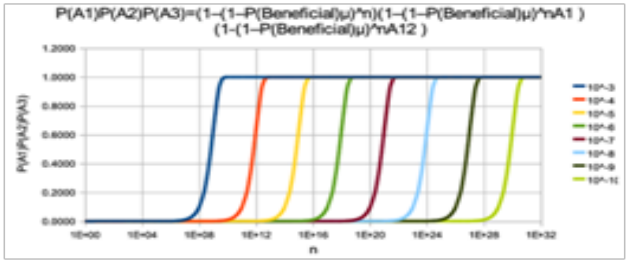

Figure 3: Probability curves for three mutations occurring as a function of n, the number of replications for various values of P(Beneficial)μ.

Figure 3 gives the probability of three particular mutations occurring as a function of the number of replications of the particular genome and yet again, with the beneficial mutation rate as a parameter. These curves give the probability that a cancer cell variant will exist in the tumor cell population with beneficial mutations to three targeted therapy or three beneficial mutations to a single targeted therapy.

7. The Interpretation and Meaning of the Probability Curves

The above probability curves give important information about the diversity of the cancer cells in a tumor. Consider the properties of the tumor when the mutation rate is 10^{-6} and a tumor DNA replication count of 10^{+6}. The probability of that particular mutation occurring from figure 1 is about 0.6. In other words, from an initial founder cell in the tumor which now has $5*10^{+5}$ descendants (assuming diploid cells) and therefore 10^{+6} genome replications, there is a high probability that every possible base substitution has occurred at every site in the DNA in some member of that population. Some of those mutations will be detrimental, others will be neutral, and still, others may be beneficial to the cancer cell and cause the failure of the targeted therapy used to try to kill those cells. The mutation rate of 10^{-6} and a tumor size of $0.5cm^3$ will have more than enough cells for there to be resistant variants already existing in that population. Even if that tumor was near the skin surface, it would be highly unlikely to identify this tumor strictly by palpation. As that tumor grows (all the variants are replicating), more mutations are being accumulated by the different variants in that population. Consider the distribution of different variants when the population size reaches $5*10^{+8}$ descendants (again assuming diploid cells) and therefore 10^{+9} genome replications.

When the population size reaches $5*10^{+8}$ (10^{+9} genome replications), from figure 2, and again with a mutation rate of 10^{-6}, we get the probability of the particular mutations occurring of 0.6. The implication of this is that every possible base substitution has again occurred on each of the variants in this population. Again, some of those mutations will be detrimental, others will be neutral, and some will be beneficial to the cancer cell to any possible selection pressure used to try to kill these cells. In some cases, a second beneficial mutation for a particular drug can occur on a member that has the first beneficial mutation or a beneficial mutation for one drug can occur on a member that already has a beneficial mutation for a second drug. And this can occur in a population that will fit in a $5cm^3$ volume.

If this analysis continues for the third mutation as illustrated in figure 3, the number of DNA replications goes to about 10^{+18} replications for a mutation rate of 10^{-6}. The magnitude of these numbers should sound familiar because 3 drug therapy is what is required to give a durable treatment of HIV.

8. Discussion

The unfortunate message of this analysis is that single drug targeted therapy for the treatment of cancer will probably not work unless the cancer is caught in the very earliest stage when the cell count is less than 100,000 cells when the mutation rate is 10^{-6}. And two-drug therapy appears to only have a reasonable probability of providing durable treatment if the population size is less than 100,000,000 (a $1cm^3$ tumor and again the mutation rate is 10^{-6}). Only when a third selection pressure is added is there a large increase in population size required for a lineage to accumulate more beneficial mutations to give resistance to three-drug therapy.

This analysis was based on an assumed mutation rate of 10^{-6}. If the tumor mutation rate goes to 10^{-8}, the number of DNA replications for one and two targeted treatments goes to 10^{+8} and 10^{+11} respectively. That would give tumor volume of about $1cm^3$ and $100cm^3$ respectively. It appears that single or even two drug targeted therapy will only be successful when the cancer is treated in the very earliest stages. This assumes that more than a single target can be identified and therapeutic agents can be developed for those targets. Oncologists have a much more difficult task than those treating infectious diseases. Both diseases involve evolving populations but the biology of the microbes associated with infectious diseases is significantly different from the cells that cause cancer which essentially is defective host cells. It is much easier to find targets in microbes than in cancer cells. It may be possible to find different selection pressures with different mechanisms of action for a single target such as done with different types of reverse transcriptase inhibitors used with the treatment of HIV. This analysis indicates that non-targeted cancer therapies such as radiation, surgery, and chemotherapeutic agents such as alkylating agents that damage normal cells may have to be used in addition to targeted therapies until multiple unique targets can be identified in cancer cells to achieve durable treatment.

9. Conclusion

Drug-resistance whether attempting to treat infectious diseases or cancers will always be a problem since mutations occur and the number of mutations depends on the number of replications of the disease-causing population. Understanding how population size relates to the formation of these mutational variants gives the correct framework in which to develop successful and durable treatment protocols. The correct understanding of the mathematical principles gives guidance and determining the number of targeted selection pressures needed in order to give durable treatment.

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