

The failure of radical treatments to cure cancer: can less deliver more?

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Abstract: All too often attempts to deliver improved cancer cure rates by increasing the dose of a particular treatment are not successful enough to justify the accompanying increase in toxicity and reduction in quality of life suffered by a significant number of patients. In part, this drive for using higher levels of treatment derives from the nature of the process for testing and incorporation of new protocols. Indeed, new treatment regimens must now consider the key role of immunity in cancer control, a component that has been largely ignored until very recently. The recognition that some drugs developed for cytotoxicity at higher doses can display alternative anticancer activities at lower doses including through modulation of immune responses is prompting a significant re-evaluation of treatment protocol development. Given that tumours are remarkably heterogeneous and with inherent genetic instability it is probably only the adaptive immune response with its flexibility and extensive repertoire that can rise to the challenge of effecting significant control and ultimately elimination of a patient's cancer. This article discusses some of the elements that have limited higher levels of treatment outcomes and where too much proved less effective. We explore observations that less can often be as effective, if not more effective especially with some chemotherapy regimens, and discuss how this can be exploited in combination with immunotherapies to deliver nontoxic improved tumour responses.

Keywords: cancer vaccines, checkpoint inhibitors, chemotherapy, immunotherapy, low-dose therapy, metronomic therapy, maximum tolerated dose, myeloid derived suppressor cells, radiotherapy, toxicity

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Introduction

Medical oncology has developed from the concept that high doses of chemotherapy are needed to eliminate every last tumour cell. With a few exceptions, such as certain leukaemias and lymphomas as well as some testicular cancers, this approach has not resulted in lasting complete cures in the majority of cancers.

Indeed, increasing the dose of standard regimens does not necessarily deliver improved outcomes as measured by regression or increased overall survival. A reduction in the quality of life is a major problem with over-treatment. Even if overt toxicity does not intervene, the majority of solid tumours will ultimately relapse. In this commentary we provide some examples of cancer treatments where lower doses of treatment can be as effective without the added toxicity of

higher doses (more can be less). Furthermore, we highlight some of the limitations of the development process that drives this approach. Until recently, the role of the immune response in delivery of cancer control and even cure has been ignored by many scientists and clinicians who have claimed all aspects of response to the specifics of their chemotherapeutic or radiotherapeutic protocols. It has now become apparent that some drugs developed for cytotoxicity at high doses may have alternate anticancer activity at lower doses. At least some of such observations are consistent with influences on the immune response. We explore observations that less can often be as effective, if not more effective (less can be more), especially with some chemotherapy regimens, and discuss how this could be applied to the burgeoning field of immunotherapy for cancer.

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Treatment of cancer (more can be less)

The early history of cancer treatments is littered with examples of initial successes leading to more aggressive, higher dosage or sustained procedures which subsequently failed to deliver improved outcomes. Surgery was the first natural treatment for cancer and was increasingly radical, especially after the availability of anaesthesia, but unfortunately this had little or no impact on local or distal spread.¹ While surgery is still the most effective modality of cancer treatment, the trend has become for more conservative interventions that retain organs and structures with associated improved body functions. Even 40 years ago, the radical mastectomies being performed on patients with breast cancer left women with deformities of the chest wall. Since then a better understanding of the patterns and likelihood of spread have allowed the use of less-mutilating simple mastectomy or simple excision followed by radiotherapy. Although combining low-volume surgery with radiotherapy was initially ridiculed by 'radicalistic' surgeons, a randomized trial organized by Bernard Fischer, to much contemporary resistance, showed that there was no benefit from radical surgery in terms of survival, recurrence or mortality, compared with local excision plus radiotherapy, in spite of the dreadful morbidity of the radical procedure.^{2,3}

While radiotherapy can successfully treat local lesions it has no effect on metastatic disease outside the treatment area. The underlying principle derives from the likelihood that most tumour cells divide faster than normal cells, although given the heterogeneity of cancer, this is not always the case. Importantly, radiation given at high doses causes ulceration, bone necrosis and marrow failure, limiting its capacity for delivering high cure rates. A therapeutic window can be exploited as the dose-response curves of the probability of tumour control and the occurrence of late effects in normal tissues using animal models show a higher threshold in normal tissues. In practice, small differences in dose in the therapeutic range can significantly affect the probability of cure and also the incidence of normal tissue injury.⁴ Extensive research has provided the knowledge base to continue to optimize tumour control and limit normal tissue injury after radiotherapy. Modern linear accelerators allow precise dose delivery to the shape of the tumour with conformal options able to deliver the dose in three dimensions, killing the cancer but avoiding sensitive normal tissues. Brachytherapy and fractionation of

external beam therapy have been optimized for the management of many common tumours. Gamma knife and cyber knife techniques have taken this concept further.

Chemotherapy initially developed from the observation that soldiers exposed to mustard gas in the First World War had atrophy of the marrow and lymph nodes. Obviously, the dose of drugs derived from 'mustine' had to be effective against the tumour without completely destroying the marrow and immune system. Chemotherapy subsequently evolved into multiple agent high-dose regimens that led to the successful treatment of metastatic testicular cancer by Larry Einhorn, using bleomycin, vinblastine and cisplatin.⁵ This combination was very toxic and importantly with regards to this article, studies doubling the dose of these drugs did not improve the initial effectiveness. Many such drugs are cytotoxic and generally the tumour response is proportional to the number of cells synthesizing DNA. However heterogeneity in cell cycle and small tumour growth fractions provide obstacles to optimal killing of the tumour. Importantly the patterns of tumour growth alter as the cancer shrinks such that an initial dosing may not be sufficient to produce a cure. This requires management of the treatment protocols as the tumour shrinks either by increasing the dose intensity (e.g. leukaemia) or through the use of alternative drugs with a different mechanism of action, and with the added potential to combat the emergence of drug-resistant tumour cells.⁶

A central problem of assessing cancer treatment effectiveness is that the measures of 'success' do not equate with tumour elimination. Thus, the smallest tumour detectable by physical or radiological means is about 1 cm in diameter and composed of about 10^9 cells. This represents about 30 doublings of an initially clonal tumour indicating a significant life history in which to produce cellular variation from a mutation frequency of 10^{-6} . While the amount of cell kill is dose dependent regardless of tumour burden, at cessation of treatment there may well be at least 10^9 residual cells, which makes detectable relapse and clonal variation almost inevitable. The recognized existence in many cancer types of tumour-initiating cells or cancer stem cells that are able to avoid the effects of radiation or chemotherapy through quiescence or a protective niche presents another formidable obstacle to cure.⁷ This exemplifies the central tenet that increasing the dose of radiotherapy and

chemotherapy does not necessarily increase the effectiveness, although the toxicity is certainly enhanced to unacceptable levels, requiring extensive supportive treatments. The dramatic impact of chemotherapy on leukaemia, lymphomas and some rare tumours, such as testicular cancer, have not translated readily to the more common solid tumours, many of which occur in older populations who are particularly sensitive to the toxic side effects of many commonly used drugs.

Other specific examples whereby dose intensification does not enhance clinical benefit include cytarabine and asparaginase.⁸ A further example is cisplatin, where high doses have no significant clinical benefit over moderate doses in a randomized in non-small cell lung cancer.⁹ Increasingly targeted drugs (e.g. palbociclib and Braf inhibitors) are being found to be as effective at lower doses or when given less frequently. Lenalidomide (cc-5013) is a good example of a drug now used regularly at a dose of 25 mg, which is a fraction of its maximum tolerated dose (MTD) dose of 160 mg.¹⁰

It is worth considering the process whereby a drug regimen becomes a licenced treatment for patients. Preclinical tumour models are used to establishing a dose response relationship and to optimize the differences in normal and tumour tissue responses. In general, a 20% dose reduction leads to a halving of the cure rate because of residual tumour cells, while in tumours with a high growth fraction, doubling of the dose can deliver a tenfold increase in tumour kill.^{11,12} It is the latter which has frequently driven the testing of higher doses to improve cure rates. While dose intensity (drug/unit time) variations and scheduling can influence the potential toxicities, those relating to the myocardium, lungs and kidneys often cannot be clinically managed.^{13,14} The dose of the drug used in the clinic is initially derived from the LD₅₀ or the dose that kills 50% of mice in a cage. This then guides the phase I trial dose where the dose is increased in trial subjects until MTD is achieved, which subsequently leads to a slight reduction in the dose for testing efficacy for phase II trials. As drugs have become more targeted this approach has become more attenuated over the last decade. Nevertheless, the fact that lower doses may be more effective when combined with other drugs or modalities, such as radiotherapy and, of increasing importance, immunotherapy, is often only appreciated after post licensing clinical experience. The advent of

biological therapies, such as cytokines and antibodies, may be particularly inappropriate for determining dosing from MTD data. Indeed, this new landscape of treatment modalities has had to be tested and 'optimized' in the context of existing standard of care (SOC) where the latter has evolved in the MTD model of approval. This raises the possibility that current SOC treatments for some cancers might benefit from re-evaluation. In particular it will be important to consider how combinations of surgery, radiotherapy and chemotherapy can influence the preservation, reactivation and stimulation of the immune response as a means of controlling or eliminating cancer. Unfortunately, the best way to optimize this approach is not immediately obvious and will need to be guided by leads from combination studies in preclinical models.

Lower-dose treatments in cancer (less can be more)

Some drugs developed for cytotoxicity at higher doses can display alternative anticancer activities at lower doses. Drug schedules given in low doses at regular intervals (daily or weekly) are referred to as metronomic. Such protocols were initially developed to help control paediatric tumours when full doses were too toxic.^{15,16} A review of the literature, by Lieu *et al.*, documented that the majority of studies reported favourable outcomes, usually in patients with heavily pretreated advanced disease, but without any significant side effects.¹⁷ The majority of metronomic regimens contain cyclophosphamide (CY) at a dose of 50–100 mg a day, although some are as low as once a week. Low-dose methotrexate is often included at 2.5 mg, twice daily. Other drugs also used include capecitabine and vinorelbine.

As the cytotoxic activity of many drugs is usually dose related it is likely that the mechanisms of action of these agents is different when used at metronomic doses. Several mechanisms that contribute to anticancer activity of CY at lower doses have been demonstrated. For example CY at low doses can exert an antiangiogenic activity and thereby contribute to disease control.¹⁸ Vascular abnormalities are a characteristic of most solid tumours and derive from raised levels of proangiogenic factors, such as vascular endothelial growth factor and angiopoietin 2. Importantly, drugs targeting such molecules do improve therapeutic responses in some part due to normalization of the tumour vasculature allowing for increased cellular

immune infiltration into tumours providing for modulation of the immunosuppressive tumour microenvironment (TME).^{17,19}

Another recognized activity of metronomic CY therapy is through immune modulation including for example the inhibition of CD4 T-regulatory cells (Tregs).²⁰ These are characterized by expression of the transcription factor FOXP3 and are a highly immune-suppressive subset of CD4 T cells that maintain immune homeostasis. Many pre-clinical and clinical studies have shown that Tregs interfere with immune surveillance of tumours compromising activation and/or function of effective antitumour immunity thereby promoting tumour progression.²¹ Targeting Tregs may be pivotal for reactivation of useful existing tumour immunity or providing an increased opportunity for the actions of other cancer immunotherapies. One approach investigated in preclinical models is the use of CY to enhance the effect of vaccines although the timing of measurable benefits may not always fit with those expected by more direct tumour-killing approaches.^{22,23} Others have reported that CY was most effective in enhancing vaccines when given one day prior to vaccination, with no effect at day seven, nor in higher doses, which were often ineffective.²⁴ These studies showed that Th-1 responses were also augmented. Importantly, several other chemotherapy agents were shown to share these pleiotropic immunomodulatory properties. Ghiringhelli *et al.* showed that a single dose of CY depleted CD4+/CD25+ cells (Tregs) and delayed the growth of colon cancer cells with subsequent immunization with tumour cells mixed with bacillus Calmette-Guérin (BCG) resulting in complete responses.²⁵ It is clear that CY can enhance the response to a wide range of vaccination protocols, but route of delivery by intravenous bolus prior to vaccination or metronomic oral (or both) route has yet to be optimized. A study of patients with metastatic solid tumours who had failed conventional chemotherapy investigated metronomic cyclo-phosphamide given twice daily, with one week on and one week off for a month or more. This was found to significantly reduce circulating Tregs with a concomitant reduction in their inhibitory functions on effector T cells and natural killer (NK) cells providing for recovery of peripheral T-cell proliferation and innate killing activities.²⁶

In summary, a metronomic regimen of CY does not only affect tumour angiogenesis but also strongly curtails immunosuppressive regulatory T

cells, favouring a better control of tumour progression. Overall these data support a CY regimen as a useful means for reducing tumour-induced immune tolerance before initiation of cancer immunotherapy. Importantly, it is clear that this beneficial activity is not present at high doses.

Our knowledge of potential low-dose effects of other drugs is only just beginning to emerge. For example, gemcitabine has been shown to modulate the TME through inhibiting myeloid derived suppressor cells (MDSCs), enhancing tumour HLA-1 expression and co-stimulatory molecule expression on antigen-presenting cells.²⁷ MDSCs promote cancer progression not only by suppressing immune responses but also by directly influencing tumour growth, differentiation and metastasis.²⁸ A randomized trial in metastatic pancreatic cancer patients showed a combination of gemcitabine with a heat-killed mycobacterial-based vaccine treatment increased progression-free and overall survival.²⁹ Other agents, such as doxorubicin, taxol and platinum may have similar effects at lower doses. Some of these drugs given at ultra-low doses can sensitize tumour cells to NK cell killing, while 5FU and doxorubicin make cancer stem cells more sensitive to gamma delta T-cell killing *via* the TRAIL apoptotic pathway.³⁰ Another mechanism of action of metronomic chemotherapy documented for other drugs is the induction of the immunogenic cell death pathway that activates both innate and adaptive immune responses. Repeated low to medium doses can induce and maintain this activity.³¹

Similar to low-dose chemotherapy, radiotherapy has been shown to have beneficial effects on the stroma and immune response when given in low doses. Low-dose radiotherapy (LDR) stimulates antioxidant capacity, repairs DNA damage and apoptosis, as well as inducing immune responses. All these aspects were reviewed and the influence of dose comprehensively discussed by Kumari *et al.*³² The exploitation of radiotherapy as a means to optimize cancer immunotherapies is thus being actively explored.^{33,34} There are several means by which radiation can act as an adjuvant to immunotherapies. The promotion of immunogenic cell death (ICD) leads to activation of antigen-presenting cells that then induce other immune cells able to attack the cancer targets.^{35,36} The radiation can also directly sensitize the tumour cells to immune effector cell killing through induction of molecular changes in the cancer cells (immune modulation).³⁷ Radiation

also directly influences the function of immune cells.³⁸ The extent of each mode of radiation action is determined by the dose and delivery scheme used (single dose or delivery of dose in smaller fractions). As summarized by Kumari *et al.*³² in the low-dose therapy range (≤ 2 Gy) the immune-stimulatory effects on tumour cells include upregulation of MHC-1 (enhanced antigen presentation), promotion of apoptosis (FAS/FasL) and recruitment of NK effectors (NKG2DL (MICA/B)) while immune cells produce more interferon (IFN)- γ (activation of CTL and Th1 bias), tumour necrosis factor (TNF)- α (induction of tumour apoptosis), interleukin (IL)-12 (promoting Th1 response), IL-2 (T cell proliferation), upregulation of CD80/86, CD28 and reduced CTLA-4 (increased co-stimulation) and reduction of macrophage produced immunosuppressive cytokines such as IL-10 and IL-1 β . However, there can also be upregulation of levels of transforming growth factor (TGF)- β and inducible nitric oxide synthase (iNOS) which can counteract positive aspects and the relative levels of influence are likely to be TME and dose dependent. Thus, appropriate LDR can exert useful anti-inflammatory activity *via* direct effects on activated macrophages while promoting Th-1 cytokine responses which contrasts with the Th-2 cytokine responses induced by higher-dose radiotherapy.³⁹ In addition, LDR affects bone marrow derived mesenchymal stromal cells so that they revert from tumour-promoting to tumour-inhibiting activity.^{32,40}

The challenges of optimizing immunotherapy

The challenge of developing improved treatment regimens must now also consider the key role of immunity in cancer control with this most likely to be through provision of optimized and integrated multimodality protocols. However, even from the first dawn of immunotherapy the siren voice that more is better has characterized attempts to develop more efficacious treatments. Thus, Coley's toxins were required to induce toxic hyperthermia to be of benefit and this subsequently limited any further development when radiotherapy machines became available. IFN- α was the first pharmaceutical-grade immunotherapy and was developed for use at high doses for metastatic melanoma. Numerous trials showed this treatment to be too toxic at high doses to develop further as an adjuvant treatment, especially when a randomized

study showed no survival benefit compared with lower, less-toxic doses.⁴¹⁻⁴³

IL-2 was first investigated as a T-cell growth factor to help improve T-cell expansion in the search for human retroviruses. Rosenberg and colleagues reported complete responses in patients with metastatic melanoma, responses which often resulted in long term 'cures'. Unfortunately, the doses used were frequently highly toxic and necessitated intensive care unit support for up to 6 weeks; this is clearly impractical in most healthcare systems. Once again, lower doses were shown to be as effective in some cases, but not as reliable in inducing complete responses. However, when used as a booster after another agent, such as a vaccine or even chemotherapy, significant responses are seen even at low doses given subcutaneously.⁴⁴ This is important as IL-2 expands recently activated T cells and, hence, does not need to be given in high doses if chemotherapy or antigen stimulation *via* other means, such as a cancer vaccine, have been given first. In other words, the toxicity of single-agent high-dose IL-2 can be avoided if given in a relevant combination at a lower dose.^{45,46}

We have recently reviewed the state-of-the-art of current checkpoint inhibitors (CPIs) in the clinic⁴⁷ and will not go into details here, suffice to say that the first CPI, ipilimumab or Yervoy, was also able to induce good clinical responses (although very few complete responses were seen). However, this came at a very high cost in terms of toxicity. Severe colitis, in particular, can be a life-threatening complication. Other CPIs, such as pembrolizumab (Keytruda) or nivolumab (Opdivo) also have significant autoimmune side effects with a slightly different and less severe profile than ipilimumab. Complete responses and clinical benefit improve when these agents are given together but unfortunately the toxicity is also more than additive, leading to early cessation of treatment (previously reviewed in detail⁴⁷).

The question has to be asked, are we giving CPIs in too high doses? Initial development of Yervoy occurred at 10 mg/kg but is now given at 3 mg/kg following dose comparative studies. The current state of the art is to combine CPIs with just about any other (often failed) immunotherapy agent and there are over 300 trials with at least 15 different drug classes in development in the clinic. Once again, added toxicity with these combinations is the main problem in combination

treatment. Is 'more can be less' appropriate here too? We would argue that this is very likely the case but, where appropriate, synergies can allow for less-toxic schedules to be developed. One such synergy that has been observed is that patients who have previously been treated with a heat-killed Mycobacterium product, IMM-101, who then progress, seem to respond quicker and more completely when given CPIs.⁴⁸ The fact that over 50% of patients have no response to CPIs alone suggests that the immune response needs to be in a primed state to respond. IMM-101 activated myeloid/macrophages in addition to the innate immune response, including NK and gamma delta T cells. A trial to investigate this possibility is about to commence (ClinicalTrials.gov identifier: NCT01559818).

Another example is the development of an optimised combination of a human papillomavirus (HPV) 16 E6/E7 synthetic long peptide therapeutic vaccination⁴⁹ with standard carboplatin and paclitaxel chemotherapy. Treatment of HPV 16 positive tumour-bearing mice with chemotherapy and vaccination improved survival significantly. It was shown that the chemotherapy directly impacted the myeloid cells systematically and in the tumour but had no effect on tumour-specific T-cell responses. In advanced cervical cancer patients, carboplatin paclitaxel was also able to normalize the concentration of circulating myeloid cells, and this was linked to improved T-cell responses. The nadir of myeloid cells, 2 weeks after the second cycle of chemotherapy, was selected as the time for vaccination. This timing was shown to be effective in patients where strong and sustained HPV16-specific T-cell responses to a single dose of the vaccine were elicited.⁵⁰ A clinical trial (ClinicalTrials.gov identifier: NCT02128126) is now in progress that is assessing the safety, tolerability and the HPV specific immune responses of different doses of the long peptide HPV16 vaccine with or without pegylated IFN- α as combination therapy with carboplatin and paclitaxel. There is some evidence that treatment of larger tumours may benefit from the use of CPIs so optimization of appropriately dosed combination approaches is awaited.

CPIs are ideal agents to enhance other immunotherapies, such as vaccines, as are the many drugs that act as immune modulators. The potential for carefully designed sequential nontoxic combinations is enormous and a logical consequence of the less is more history of cancer treatment

development. Meanwhile, it is going to be very important to modulate the dosing of a CPI or another immune modulator such as lenalidomide or chemotherapeutic agents. Too often promising combinations have been discarded because of added toxicity when doses of all agents should be lowered appropriately. Moreover, it may be possible that a specific sequence of treatments may synergize, as opposed to adding treatments together. We have previously reported that lenalidomide and pomalidomide given before vaccination greatly enhance responses⁵¹ whereas when given after vaccination there is no benefit.⁵²

In conclusion, it may be much more effective to work out scientifically logical sequential therapy, as opposed to just adding more modulates together. Biological agents generally show effectiveness over bell shaped rather than exponential dose-response curves. Indeed, a scientific example that less can deliver *much* more.


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