Immunogenic Cell Death: Bench Comes to Bedside... With Caveats

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Immunologic therapies for cancer wax and wane in popularity. Interest is now waxing for monoclonal antibody-based checkpoint inhibitor therapy because of incredible responses in patients with advanced lung, kidney, and melanoma cancers. In addition, the use of oncologic viruses such as IMLYGIC (talimogene laherparepvec or T-VEC) has spurred a renewed enthusiasm for such viruses as immunologic agents. Radiation and phototherapy also seem to drive immune responses that cause untreated lesions to regress (the abscopal effect). Checkpoint inhibitors are now standard of care in first line treatment of Non-Small Cell Lung Cancer. However, recent data indicate that checkpoint inhibitor therapy appears to be most successful in patients whose malignancies have a high Tumor Mutational Burden to create neoantigens, numerous inflammatory and immune cells that infiltrate tumors to make them “Hot,” and evidence of an immune response by the expression of PD-L1, a checkpoint inhibitor ligand.

Current analyses indicate that less than half of all cancers are associated with these three characteristics. As a result, the overall response to these inhibitors is less than 50 percent. In colorectal carcinoma, where the “Hot” category is limited to 12-15 percent of these cancers that are microsatellite unstable, the response rate to checkpoint inhibitors is seven percent and generally limited to the microsatellite unstable subset.

The enhanced response rate to T-VEC when given intralesionally along with persuasive preclinical data suggest that certain cancers, like colon cancer, may show a much higher response rate if the therapy is administered intralesionally as compared to the usual parenteral administration. A resulting higher Immunogenic Cell Death (IHC) could be a great benefit to the cancer patient.
T-VEC for intralesional treatment of metastatic cutaneous melanoma is an important example of why immunology may be important. About a quarter of patients have regression of uninjected lesions as well as those who have been injected when T-VEC is injected intratumorally into cutaneous metastases of melanoma. FDA has considered this as evidence of possible immune-mediated regression. It is difficult to demonstrate that these unexpected regressions result from an immune response, and, in some cases, may be delayed responses to prior therapies. Is there a possible explanation for these phenomena where a treatment may cross-prime adaptive immune responses to tumor antigens?

Abbreviations

C\text{MAX} \\
Maximum concentration of an agent \textit{in vivo}, usually in the central compartment \\
DACH \\
1,2-diaminocyclohexane, the carrier ligand for Oxaliplatin \\
ER \\
Endoplasmic Reticulum \\
IC\text{50} \\
50 percent Inhibitory Concentration of a drug measured \textit{in vitro} in a cytotoxicity assay \\
ICD \\
Immunogenic Cell Death \\
T-VEC \\
Oncolytic virus talimogene laherparepvec \\

ICD causes cells to undergo a dignified death. As they die, they release peptides and antigens with mediators that cause inflammation and stimulate innate and adaptive immunity and cross-primes to tumor antigens. Caspase-dependent apoptosis caused by standard chemotherapy is non-immunogenic and occurs around the concentrations at which chemotherapeutic agents kill malignant cells, i.e., generally within 1-3 times the IC\text{50} for a drug (Figure 1). In apoptosis, cells die quietly without causing inflammation. However, ICD generally requires a higher concentration of drug or death induction. ICD was initially identified by Casares and colleagues at the Institut Gustave Roussy. They exposed murine rectal carcinoma CT26 to 10-25 times the IC\text{50} of Doxorubicin for 24 hours and then transplanted the cells into syngeneic BALB/c mice and found that the dying CT26 cells generated an immune response mediated by CD8+ cytotoxic T-cells. These cytotoxic T-cells rejected a viable tumor cell challenge and displayed both memory and antigen specificity.

Studies soon followed that demonstrated that other anthracyclines, Oxaliplatin (but not Cisplatin), a few other chemotherapeutic agents, oncolytic viruses, radiation therapy, and phototherapy could also induce similar specific adaptive immunity in preclinical models. The ICD effect generally required more than three
times the $IC_{50}$ for that chemotherapeutic agent in standard *in vitro* cytotoxicity assays.

Figure 1. Immunogenic Cell Death (ICD) Occurs at Drug Concentrations Higher than the $IC_{50}$. ICD requires the externalization of the ER chaperone Calreticulin as the start of the immunization process. This occurs at concentrations higher than those of the $IC_{50}$ *in vitro* that causes caspase-dependent non-immunogenic cell death. The $C_{MAX}$ may be a little higher *in vivo* but, at least for Oxaliplatin, has a short 0.25-0.3 hour half-life and reflects only the drug in the central compartment of the circulation, not the concentration in metastases or the liver. The Zone of ICD, based on the externalization of Calreticulin in human or mouse colorectal carcinomas, is depicted above and in some reports may extend as high as 300 µM. However, death may occur so quickly at those higher concentrations that Calreticulin may not be externalized. $C_{MAX}$ depicts the $C_{MAX}$ reported in the central compartment of an isolated hepatic perfusion loop at the maximum tolerated dose of 40 mg/M2. $C_{MAX}$ from 85-130 mg/M2 BSA Oxaliplatin *in vivo* depicts the average concentration of active Oxaliplatin in liver metastases achieved during one hour of isolated liver perfusion. These results suggest that regional therapy may achieve the concentrations needed to drive ICD. They also may explain why ICD is not frequently seen during systemic therapy with ICD inducers like Oxaliplatin.

The mechanism of ICD invokes primordial cellular responses to pathogens as well as modulation of cell survival pathways. Investigators soon found that Oxaliplatin and the anthracyclines induced ER stress through separate mechanisms that cause a series of changes which appear common to ICD. First, ICD agents either cause oxidative stress or the Unfolded Protein Response that in turn causes ER stress. Oxaliplatin and other ICD inducers may not activate the Unfolded Protein Response but directly cause oxidative stress that activates eukaryotic translation initiation factor 2 alpha kinase 3 (EIF2AK3 or PERK), which in turn phosphorylates and inhibits eIF2a, shutting down general protein synthesis. With activation of PERK, there is also activation of another ER stress molecule, BECLIN-1 (BECN1 or ATG6), that initiates an autophagy response. Depending on the strength of the signal autophagy, it keeps the cell alive for a time and, paradoxically, supports the apoptotic pathway by activating Caspase 8 and subsequently the extrinsic or intrinsic death pathways. During this focused activation of an apoptotic response, Calreticulin, an endoplasmic reticulum (ER) protein that normally chaperones nascent proteins during synthesis and post-translational modification within the ER, herds nascent proteins to the external surface of the cell by anterograde ER-Golgi traffic with exocytosis.
The externalization of Calreticulin with the ER luminal contents occurs before of membrane permeability and the externalization of Annexin V. Also, this exocytosis is associated with the release of ATP along with Danger Associated Molecular Pattern (DAMP) molecules, such as HMBG1 and HSP70. Once outside the cell, Calreticulin serves as an “eat me” signal with ATP that attracts dendritic and other antigen-presenting cells to the dying cancer cell. The DAMPs then provide “take me” signals that load dendritic cells and activate their ability to activate naive T- and B-cells. As shown in Figure 1, the concentration to induce ICD activity is generally greater than the $C_{MAX}$ for such chemotherapeutic agents as Oxaliplatin.

This process of ICD makes “Cold” tumors “Hot.” In our pre-clinical experience, ICD caused a four-fold increase in infiltrating CD3+ T-cells that were naive with decreased expression of PD-1 on CD8+ T-cells compared to infiltrating CD8+ T-cells in untreated tumors that were PD-1+ and exhausted. ICD in the established CT26 tumors after a single intratumoral injection of Oxaliplatin and oncolytic virus inhibited tumor growth in all mice with complete regression in 40 percent of mice, cytotoxic lymphocytes in draining nodes and transplantation resistance that rejected a viable tumor cell challenge. All with just a single intratumoral injection and without the addition of checkpoint inhibitors.

However, there is a major caveat to the induction of ICD. Since ICD requires multiples of the IC$_{50}$ for the inducing drug, it seems unlikely that standard systemic chemotherapy will actually induce ICD in the majority of patients. As shown in Figure 1 for Oxaliplatin, the $C_{MAX}$ after the systemic administration of either 85 or 130 mg/M$^2$ is barely into the region that causes ICD in tumor tissue. The $C_{MAX}$ is measured in the central compartment of blood where the half-life is 15-25 minutes. The hepatic clearance from the central compartment is not described in the literature for systemic administration, so it is difficult to predict what the intratumoral concentration of Oxaliplatin will be.

A small Phase 1 trial of isolated hepatic perfusion in Pittsburgh achieved a $C_{MAX}$ three times that of systemically administered Oxaliplatin, with a concentration of Oxaliplatin in metastases that was just inside the range of Oxaliplatin concentration that causes ICD (Figure 1). Oxaliplatin, like other agents, has a limited time of activity before its effectiveness is lost. It and its metabolites of mono and dichloro DACH platin and diaquo DACH platin are active for only four hours before being inactivated by conjugation and protein binding. Thus, systemic administration of Oxaliplatin would need to be increased by more than three-fold to induce ICD within liver metastases. Since other cytotoxic ICD inducers like Oxaliplatin are near their maximum tolerated dose, it is not likely that systemic administration will stimulate an immune response to the tumor by itself that causes regression of an established disease.
Regional delivery may be the answer. In seven patients in the Pittsburgh Phas trial with unresectable colorectal carcinoma liver metastases there was one complete response, one progressive disease, one stable disease, and four partial responses. With techniques like transarterial chemoembolization or image-guided intratumoral injection, it is likely that the high intratumoral concentration of Oxaliplatin and similar drugs may be obtained without causing systemic toxicity. Work with nanoparticles that target tumors with a high enough concentration to activate ICD without causing systemic toxicity are also likely to cross-prime patients’ immune responses to their tumors.

In summary, ICD is a product of the bench, but its usefulness needs to be put into the context of clinical care. ICD requires intratumoral concentrations of inducers that may seem too high for systemic administration. However, the potential benefits of ICD may be reaped with advances in targeted delivery of ICD inducers to malignant lesions. Finally, if improvements in regional therapy with ICD inducers makes “Cold” tumors “Hot,” then nearly 60 percent of all cancer patients will become candidates for checkpoint inhibitor therapy.