MELANOMA IN FOCUS

Current Developments in the Management of Solid Tumor Malignancies

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Intermittent Dosing in Melanoma

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H&O What are the limitations of continuous drug dosing in melanoma?

MM We know that approximately half of melanomas have mutations in *BRAF* that activate the mitogen-activated protein kinase pathway. Agents that block the BRAF V600E enzyme, or its downstream targets MEK1 and MEK2, can have a dramatic effect on patients with advanced melanoma, but they do not provide a cure. The tumors often respond remarkably well at first, but then the response stops and the tumors come back in a drug-resistant form, so that continuous use of the drug provides little or no additional benefit.

The second limitation is the potential for toxicity. Although BRAF inhibitors are substantially less toxic than many conventional agents used in cancer chemotherapy, they do have some toxicity—and continuous dosing can sometimes lead to cumulative toxicity. There also can be issues of adherence with a pill that needs to be taken every day.

H&O How does drug resistance to BRAF inhibitors develop in melanoma?

MM There are perhaps a dozen mechanisms by which melanoma can evade the effects of these BRAF inhibitors, as has been documented in numerous papers. One of these mechanisms is that BRAF can be expressed in the cell at a higher level through gene amplification or overexpression, so that it takes more of the drug to inhibit the enzyme to the same extent. Because of dose-limiting toxicities, the patient cannot receive enough of the drug to be effective. Gene amplification as a mechanism of drug resistance was studied at Stanford in the 1970s by Bob Schimke, who used Chinese hamster ovary cells in tissue culture to determine that amplification of the gene encoding dihydrofolate reductase (DHFR) caused resistance to methotrexate. This is the same type of resistance that can occur with BRAF-specific drugs in *BRAF*-mutated melanoma.

H&O Could you describe your own research on drug resistance in melanoma?

MM My laboratory collaborated with Darrin Stuart's laboratory at the Novartis Institutes for Biomedical Research to study drug resistance in patient-derived xenografts; this work appeared in *Nature* with Meghna Das Thakur as the first author. We started by implanting human *BRAF*-mutated melanoma tumors into mice without an immune system. The tumors shrank away to almost nothing when they were treated with vemurafenib (Zelboraf, Genentech/Daiichi Sankyo), which is what we expected to see. When we continuously dosed the mice, however, we eventually saw emergence of drug-resistant tumors caused by elevated expression of BRAF V600E—which is exactly what is seen in some melanoma patients.

We observed that when we took the drug away from the resistant tumors, all of that extra BRAF V600E turned out to be deleterious to the cells and the tumors went into regression. This remarkable finding told us 2 things. First, these tumors were not just drug resistant; they were drug addicted, and needed the continuous presence of the drug in order to proliferate at their maximum capacity.

Second, they were suffering from a phenomenon I refer to as "oncogene overdose"—in the absence of drug, they had too much of the thing to which they were addicted, that is, the BRAF V600E oncoprotein. Therein lay the fundamental observation, which was that *BRAF*-mutated melanomas are sensitive to the magnitude of pathway activation. Hence, there is a Goldilocks effect: too little or too much BRAF V600E pathway activation is bad for the tumor. However, in the Goldilocks zone, the amount of BRAF V600E pathway activation allows the tumors to find their maximum capacity for propagation.

We realized that these drug-resistant tumors had a growth disadvantage if the drug was absent, which we referred to as a "fitness deficit." After some discussion with our medical oncology colleagues, we developed the idea that we might be able to kill off the drug-sensitive cells in the first round of treatment, and then create a fitness deficit in the resistant cells by discontinuing the drug using an intermittent drug dosing protocol.

When we experimented on our xenograft mice, we found that all of the mice that received continuous dosing of vemurafenib for 150 days developed drug-resistant disease. By contrast, tumors in the mice that were dosed in a 4-weeks-on, 2-weeks-off pattern regressed and grew, regressed and grew. The remarkable finding was that they never developed drug-resistant disease over the course of our analysis. That finding suggested a relatively simple way to alter our use of an existing therapy in an attempt to prolong the durability of response in patients with melanoma.

H&O What evidence exists that this approach might be effective in humans?

MM The first suggestion that this might be feasible in humans comes from a very elegant case report by Callahan and colleagues—from Paul Chapman's group at the Memorial Sloan Kettering Cancer Center—that was published in the *New England Journal of Medicine* in 2012.

One of the curious characteristics of BRAF inhibitors is that they have a paradoxical ability to promote tumorigenesis under some circumstances. Indeed, it is somewhat embarrassing that the drugs we are developing to block *BRAF*-mutated melanoma also have the ability to promote the growth of incipient tumor cells with a *RAS* mutation. For example, we have seen these agents promote the growth of relatively benign skin tumors that are completely unrelated to the melanoma.

In the Callahan study, the researchers noted that a patient in their clinic who was being treated for a *BRAF*-mutated melanoma developed an *NRAS*-mutated chronic myelomonocytic leukemia. They hypothesized that the *BRAF* inhibitor shrinking the melanoma was also promoting the growth of the patient's leukemia, and that taking the patient off the agent temporarily might make the leukemia regress. By using intermittent cycles, they might be able to keep both diseases in check at the same time.

In their case report, the researchers showed that this approach was, in fact, feasible. When the patient was on the BRAF inhibitor, the melanoma would shrink and signs of leukemia in the blood would increase. When the patient was off the agent, the melanoma would start to grow again and signs of leukemia would decrease. Although the researchers had not undertaken intermittent dosing for the purpose of preventing drug resistance, their experience demonstrated the feasibility of this approach.

H&O What other research has been done on the effect of discontinuing treatment on tumor growth?

MM After we discovered this phenomenon in our research laboratory work, we were very keen to talk to medical oncologists who had participated in clinical trials for patients with *BRAF*-mutated melanoma to find out if anyone had seen anything like this in patients in clinical trials. We were very fortunate to meet Rosalie Fisher and her colleagues from the Royal Marsden Hospital in London at the 2012 Society for Melanoma Research meeting in Los Angeles. They were presenting results on 42 patients from the BRIM (BRAF Inhibitor in Melanoma)-3 vemurafenib trial whose disease had become resistant to the drug. The researchers reviewed computed tomography scans, taken before and after stopping vemurafenib, for 19 of these patients. Remarkably, they found that although the drug resistant tumors grew aggressively while the patients were on the drug, this growth was less aggressive in 14 patients after the drug was discontinued. Indeed, in some cases they saw melanoma regression following cessation of vemurafenib therapy. That suggested to us that what we had seen in the research laboratory might have a counterpart in the clinic. Moreover, Roger Lo, a colleague at the University of California, Los Angeles, confirmed our observations on oncogene overdose using cell culture–based model systems in which resistance to combined inhibition of BRAF V600E and MEK1/2 was selected in culture.

H&O What research is ongoing on intermittent dosing?

MM Several studies are looking at intermittent dosing. For example, Paul Chapman and colleagues at Memorial Sloan Kettering Cancer Center and Toni Ribas and colleagues at UCLA are conducting clinical trials of the BRAF inhibitor LGX818 in patients with *BRAF*-mutated stage IV or unresectable stage III melanoma to determine if

intermittent dosing can delay the onset of drug resistance (NCT01894672 and NCT02263898).

Alain Algazi, who is one of my colleagues at UCSF, is the principal investigator for the SWOG (Southwest Oncology Group) S1320 study (NCT02196181). This study is looking at intermittent vs continuous dosing with a combination of dabrafenib (Tafinlar, GlaxoSmithKline) and trametinib (Mekinist, GlaxoSmithKline) in patients with unresectable stage III or IV *BRAF*-mutant melanoma, to determine whether intermittent dosing with these drugs leads to a more durable response than continuous dosing. The schedule for intermittent dosing is 1 week on followed by 3 weeks off and then 4 weeks on.

H&O What results are you expecting to see?

MM The jury is very much out; I would say there is a reasonable chance that this strategy will work, but it is by no means a sure thing. One of the challenges in the S1320 study is that trametinib has a very long half-life. Will the 3-week drug holiday be long enough to impose the necessary negative selection on the drug-resistant melanoma cells? We simply do not know. Another challenge is that there is no practical way to treat individuals using a tailored pulsed protocol; the schedule needs to be standardized for the purposes of evaluation and replication.

Perhaps the most daunting challenge is the enemy of all cancer therapy: the fundamental heterogeneity that lives within any given tumor. Roger Lo has profiled the mechanisms of resistance that emerged in each of the individual lesions from individual patients at the UCLA melanoma clinic. He has observed a stunning heterogeneity of resistance mechanisms evolving in different drug-resistant tumors from a single patient. This likely reflects the fact that each metastasis emerges from one or a small number of cells and is selected for optimal growth in its own unique microenvironment. Hence, a melanoma brain metastasis is growing in a different microenvironment than a liver, kidney, or visceral metastasis. Given the differences between these metastases, it may not be reasonable to expect that each lesion will conform to the same intermittent dosing paradigm. If a patient has 25 lesions and 6 of them respond just as we had hoped, that would still leave 19 lesions that do not respond as hoped—and no extension of lifespan or progression-free survival. So the chances of failure are substantial.

The other roadblock is that cancer cells are likely to be able to evolve to resist therapy, regardless of how the therapy is applied. If we kill cancer cells using continuous dosing, we select for mechanisms of resistance that make the cells prosper when faced with continuous dosing. If we kill cancer cells using intermittent dosing, we may end up selecting for mechanisms of resistance that make the cells prosper when faced with intermittent dosing. Again, this speaks to the fact that moving from the relatively well-controlled scientific environment of the research laboratory into clinical trials in melanoma patients reveals substantial new complexities that are going to impinge upon the likelihood of success.

If the trials of intermittent therapy in metastatic melanoma that are being conducted now fail, will that be the end of the approach? Or will we detect clues that this approach might work if given earlier in the course of the disease? The best use of intermittent dosing might actually be in patients with relatively high-risk disease that cannot be resected, but is limited to 1 substantial nodule of disease that has not metastasized floridly. Another possibility might be to employ intermittent dosing in the adjuvant setting, although neither of these scenarios is without complications in clinical trial design.

H&O Are there any circumstances under which oncologists should be giving BRAF inhibitors intermittently to their patients outside of clinical trials?

MM There is no strong scientific rationale for giving intermittent dosing specifically to prevent the onset of drug-resistant disease. As the Callahan/Chapman paper demonstrated, however, it may be reasonable to try intermittent dosing in a patient who is taking a BRAF inhibitor for melanoma and has growth of an underlying malignancy such as a *RAS*-mutated leukemia.

Apart from that, the most common reason for drug holidays is to manage cumulative toxicities. Until the results of the various clinical trials have been reported, there is no compelling rationale for doing intermittent dosing to prevent resistance at this time.

Suggested Readings

Callahan MK, Rampal R, Harding JJ, et al. Progression of RAS-mutant leukemia during RAF inhibitor treatment. *N Engl J Med.* 2012;367(24):2316-2321.

Chen Z, Shojaee S, Buchner M, et al. Signalling thresholds and negative B-cell selection in acute lymphoblastic leukaemia. *Nature.* 2015.

Das Thakur M, Salangsang F, Landman AS, et al. Modelling vemurafenib resistance in melanoma reveals a strategy to forestall drug resistance. *Nature.* 2013;494(7436):251-255.

Fisher R, Ali Z, Edmonds K, Turajlic S, Gore M, Larkin J. Clinical behaviour of metastatic melanoma after progression on vemurafenib [Society for Melanoma Research]. *Pigment Cell Melanoma Res.* 2012;25(6):856.

Schimke RT, Kaufman RJ, Nunberg JH, Dana SL. Studies on the amplification of dihydrofolate reductase genes in methotrexate-resistant cultured mouse cells. *Cold Spring Harb Symp Quant Biol.* 1979;43(pt 2):1297-303.

Deuker MM, McMahon M. Oncogene addiction and overdose: intermittent treatment in models of drug-resistant BRAF-mutated melanoma. *The Melanoma Letter.* 2014;32(1):8-11.