AWARD NUMBER: W81XWH-17-1-0350

TITLE: Prognostic Biomarkers in Active Surveillance: Parsing Risk in Early Stage Prostate Cancer

PRINCIPAL INVESTIGATOR: David J. Einstein, MD

CONTRACTING ORGANIZATION: Beth Israel Deaconess Medical Center

Boston, MA 02215

REPORT DATE: August 2018

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command

Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
August 2018	Annual	01 Aug 2017 - 31 Jul 2018

4. TITLE AND SUBTITLE

Prognostic Biomarkers in Active Surveillance: Parsing Risk in Early Stage Prostate Cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-17-1-0350

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

David J. Einstein, MD

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Beth Israel Deaconess Medical Center
330 Brookline Avenue
Boston, MA 02215

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

While a majority of prostate cancers (PCa) remain clinically insignificant, some have the potential to metastasize and become lethal and therefore merit intervention. Thus, identification of men most at risk and most likely to benefit from therapy is a major clinical and public health challenge. Recent evidence suggests that the clinical-pathologic criteria used to assess eligibility for active surveillance and to define progression requiring intervention do not capture molecular changes that may more accurately predict progression of tumors from indolent to aggressive. Based on published results and preliminary data, we hypothesize that truly indolent Gleason pattern (Gp) 3 tumors are a molecularly distinct subset from potentially aggressive Gp3. During this period, we found significant differences in somatic copy number alteration (SCNA) landscape between tumor foci from isolated Gp3 versus Gp3 in close proximity to Gp4 in prostatectomy specimens. We also evaluated circulating tumor DNA (ctDNA) as a biomarker in perioperative plasma samples from patients undergoing prostatectomy for intermediate/high-risk disease but so far have not been able to reliably identify tumor mutations in ctDNA.

15. SUBJECT TERMS
Prostate cancer, active surveillance, biomarkers

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	15	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Page

1. INTRODUCTION

AS has emerged as an approach for sparing men the morbidity associated with primary therapies for indolent PCa, while closely monitoring them for disease progression that would require such therapy before the development of lethal disease. However, while it appears that deferring therapy does not have major impact on survival, there are concerns that the delayed therapy may negatively impact survival in a subset of men, and may in some cases lead to more aggressive local therapy than would have been indicated on initial discovery. Conversely, concerns that an aggressive tumor was missed on biopsy or may emerge during surveillance lead many men with indolent disease to opt for RP or radiation therapy (with approximately half of patients on AS programs eventually undergoing primary therapy). Therefore, more refined methods are needed to separate patients at low risk for progression, who should be spared morbidity of primary therapy and perhaps even serial biopsies, from those at higher risk for progression who need more intensive management and should possibly proceed directly to primary therapy. To address this need, our purpose is to identify features that identify candidate AS patients whose tumors are at increased risk of progression, or of having an undetected higher grade tumor. Based on published results and our preliminary data, we hypothesize that truly indolent Gp3/Gs6 tumors are a molecularly distinct subset and will have a relatively silent SCNA landscape, while potentially aggressive Gp3 tumors will have extensive SCNA including losses in established tumor suppressor genes. This hypothesis will be tested in Aim 1 by examining the landscape of SCNA in indolent Gs3 from AS patients who have not progressed for >5 years, versus in Gp4-associated Gp3. Our second hypothesis is that indolent Gp3/Gs6 PCa will have very low or undetectable levels of ctDNA, while potentially aggressive tumors will have higher levels that will also increase over time (which may reflect greater tumor volume, increased cell turnover, or micrometastatic disease). This hypothesis will be tested in Aim 2 by initially identifying the clonal *TMPRSS2:ERG* breakpoint in a series of RP specimens from men with high-risk PCa. We will then construct breakpoint-specific primers to detect and quantify ctDNA containing the breakpoint from plasma banked prior to RP.

2. KEYWORDS

Prostate cancer, active surveillance, biomarkers

3. ACCOMPLISHMENTS

What were the major goals of the project?

Aim 1. Determine the somatic copy number alteration (SCNA) landscape of Gleason pattern 3 from men undergoing active surveillance.

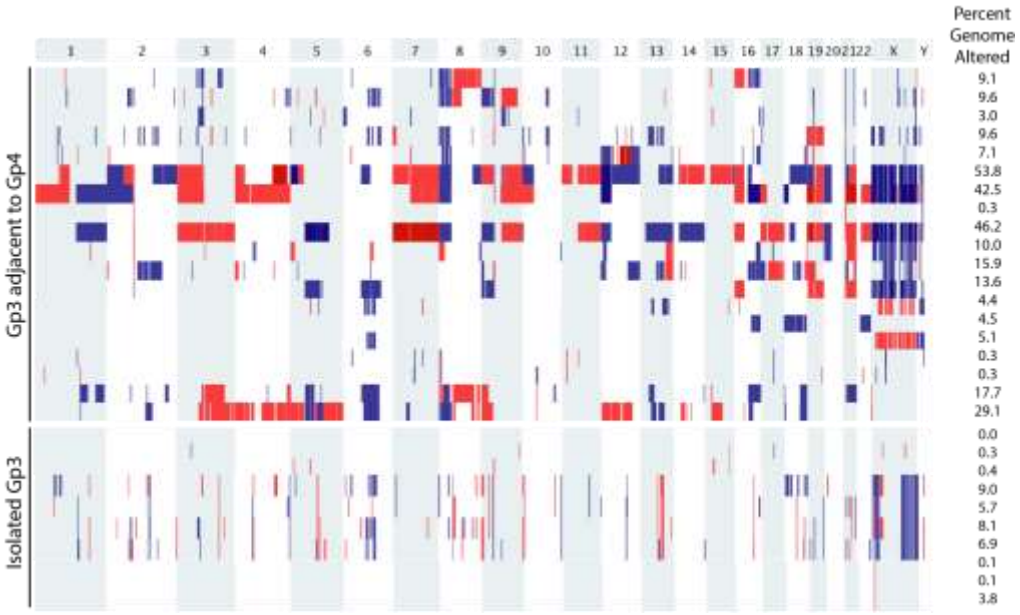
Aim 2. Determine whether circulating tumor DNA prior to radical prostatectomy is a biomarker of aggressive PCa.

What was accomplished under these goals?

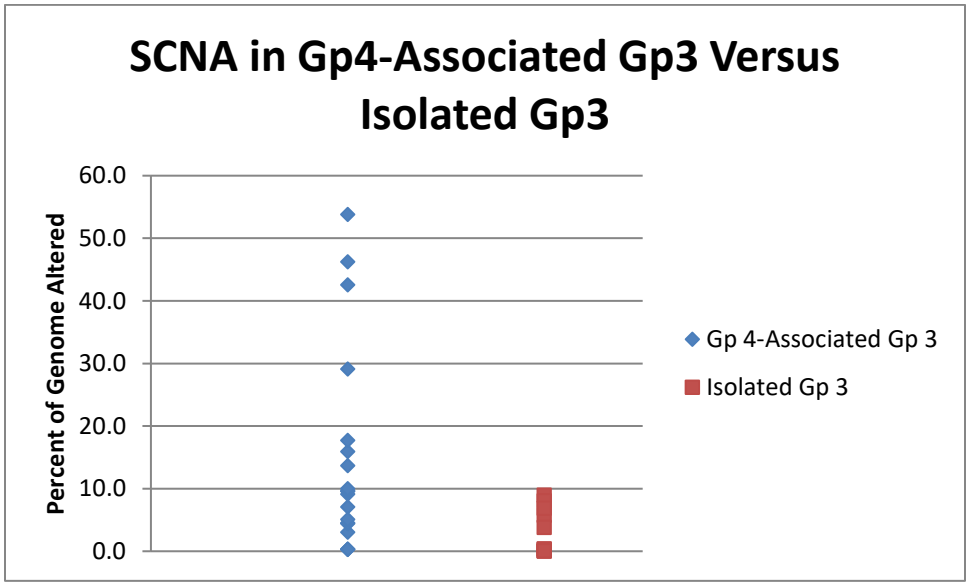
Aim 1:

Together with Dr. Adam Sowalsky at NCI, we have performed whole-exome sequencing (WES) on a cohort of patients who underwent radical prostatectomy and had Gleason pattern (Gp) 3 and Gp 4

prostate adenocarcinoma. We performed WGS separately on micro-dissected foci of Gp 3 and Gp 3 in close proximity to Gp 4 (Gp 4-associated Gp 3). We are now performing analysis of SCNA in the Gp 3 foci and Gp 4-associated Gp 3 foci. Preliminary data appear to show substantial difference in the percentage of genome altered in Gp4-associated Gp 3 foci versus isolated Gp 3 foci (figures 1a and 1b), and the median percent genome altered is significantly different between the two groups ($p<0.05$, t-test). Thus, the SCNA landscape of Gp 4-associated Gp 3 foci appears to differ from that of isolated Gp 3 foci. The former is clonally related to the adjacent Gp 4 (Sowalsky AG et al., Clin Cancer Res 2017) and therefore may have more metastatic potential than the isolated Gp 3 foci, so SCNA may correlate with metastatic potential.



Figures 1a and 1b: Heat map (1a) and scatter plot (1b) showing percent of genome altered. Heat map x-axis is chromosome number.



However, further work will be needed to evaluate SCNA as a prognostic biomarker regarding probability of clinical progression on active surveillance. To address this question, we will perform WES in tumors from patients on active surveillance (AS) who either had indolent disease over time or who ultimately progressed and required definitive therapy. This will allow us to evaluate SCNA as a biomarker for progression versus stability on AS. We will need to analyze sensitivity and specificity in order to present a receiver-operating characteristic curve and calculate area-under-the-curve to evaluate this as a biomarker. There is likely to be overlap in SCNA between the two populations, in which case this may not be a binary biomarker and so we would need to determine risk at different levels of genomic alteration.

Aim 2:

We have performed ctDNA sequencing for six patients with intermediate-to-high-risk prostate cancer who underwent radical prostatectomy and had pre-surgery and post-surgery plasma collections. In these six patients, we have detected ctDNA for only one patient and with high allele fractions suggesting potential false positive signal or clonal hematopoiesis.

Patient	Pre-RP	Post-RP 1	Post-RP 2	Post-RP 3	Post-RP 4	Post-RP 5
001	No ctDNA	No ctDNA	No ctDNA	No ctDNA	No ctDNA	
*002	1/12 targets positive	1/12 targets positive	1/12 targets positive			
003	No ctDNA	No ctDNA	No ctDNA	No ctDNA	No ctDNA	No ctDNA
004	No ctDNA	No ctDNA	No ctDNA	No ctDNA	No ctDNA	
015	No ctDNA	No ctDNA	No ctDNA	No ctDNA		
040	No ctDNA	No ctDNA	No ctDNA	No ctDNA		

*positive target was chr19:7104596C>G at high allele fractions suggesting potential false positive signal or clonal hematopoiesis

In two patients with intermediate-risk disease with defined TMPRSS2:ERG breakpoints (genomically confirmed), we were not able to identify the breakpoint in plasma by PCR. This work will need to be expanded but may highlight a limitation in our plan to use TMPRSS2:ERG breakpoints to identify ctDNA.

We have also looked for patients in this cohort who have known biochemical recurrence following their radical prostatectomy and have identified several. We will investigate whether any of these cases have detectable ctDNA in peri-operative plasma, as this could serve as a biomarker for recurrence after surgery for intermediate/high-risk disease. (In addition, if ctDNA cannot be detected in peri-operative

plasma in these cases of known recurrence, then ctDNA is unlikely to be a good prognostic biomarker for aggressive disease in any setting.) In one patient, we have obtained plasma just prior ot the start of salvage radiation, which will allow us to compare whether ctDNA is present perioperatively and/or at the time of biochemical recurrence, and, if ctDNA is detectable at both timepoints, to correlate the genomic alterations identified.

What opportunities for training and professional development has the project provided?

This project has offered a number of opportunities for my training and professional development. My overall goal is to advance my career as a translational physician-scientist in the field of prostate cancer. The salary support offered by this award has protected time to pursue a number of projects ranging from exploratory pre-clinical work to phase II clinical trials.

My first exploratory project is a study of neoantigens in localized prostate cancer. I am working together with immunology experts as well as Drs. Balk and Bhasin to identify tumor neoantigens in diagnostic core biopsies from patients undergoing radiation and hormonal therapy for intermediate-to-high-risk localized prostate cancer. I have then collected serial blood specimens with the goal of identifying whether any T cells are present that are capable of recognizing the identified tumor neoantigens.

In addition, I have set up a rapid-autopsy protocol and have successfully conducted four rapid autopsies. With tissue obtained from these autopsies and prior tissue obtained during these patients' clinical care, we are performing investigations of tumor heterogeneity and alterations in androgen receptor as well as genomic alterations in response to treatment.

Finally, I have had the opportunity to design two phase 2 clinical trials. One involves an inhibitor of polo-like kinase 1 (PLK1) in combination with abiraterone for metastatic castration-resistant prostate cancer. The other involves the PD-1 inhibitor nivolumab for patients with high-risk biochemically recurrent prostate cancer. I have written protocols and successfully obtained IRB approval for both trials. The PLK1 inhibitor trial is currently open and accruing patients. The nivolumab trial is in the activation phase.

How were the results disseminated to communities of interest?

Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?

As described above, we will perform WES in tumors from patients on active surveillance (AS) who either had indolent disease over time or who ultimately progressed and required definitive therapy. This will allow us to evaluate SCNA as a biomarker for progression versus stability on AS. We will also evaluate

ctDNA in patients known to have experienced recurrence after prostatectomy to see if this can serve as a prognostic biomarker.

Meanwhile, I will continue to work on the trials described above. We will accrue patients to both trials and collect specimens for correlative/exploratory studies. For our neoantigen study, I will work with Drs. Balk and Bhasin to identify likely tumor neoantigens and evaluate whether there are T cells in our collected blood samples capable of recognizing them. I will also continue work on the rapid autopsy protocol to expand this tissue bank and analyze the collected tissues.

IMPACT

What was the impact on the development of the principal discipline(s) of the project?

Nothing to Report.

What was the impact on other disciplines?

Nothing to Report.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Nothing to Report.

CHANGES/PROBLEMS

Changes in approach and reasons for change

Aim 1: As noted above, we will expand our SCNA studies as planned into patients who are on AS and have either indolent disease or disease that ultimately progresses. However, we are also performing statistical analysis to determine whether this approach is likely to be limited. Of note, following the submission of this project, a report was released containing analysis of whole-genome and whole-exome sequencing from several hundred localized, non-indolent prostate tumors (Fraser M et al., Nature 2017). Of note, a low overall somatic copy number variation (SNV) burden was observed, with a median of 0.53 somatic SNVs per million base pairs across all tumors. SNV burden was significantly elevated in tumors with Gleason pattern 4, although many Gp 4 tumors had low SNV burden, limiting the utility of SNV burden for predicting Gp. (It is still possible that Gp 4 tumors with low SNV burden actually behave less aggressively than Gp 4 tumors with high SNV burden.) The only gene in which SNVs were prognostic was ATM, which occurred at a rate of only 1.75%. Thus, we will use these data and our own to estimate an appropriate number of AS cases to evaluate and at what point this approach would be considered statistically futile.

If we find that there is too much overlap in SCNA between our indolent versus aggressive cohorts, then this may be limited as a biomarker. Another hypothesis worthy of investigation is that the immune microenvironment of indolent prostate cancer may differ from that of potentially aggressive prostate cancer, and that the effectiveness of the immune response is responsibility for differences in clinical outcome of histologically similar cancers. Therefore, in this case, we will consider investigation of the immune microenvironment of AS patients with indolent versus progressive disease using IHC studies to evaluate tumor-infiltrating or peri-tumoral immune cell subsets.

Actual or anticipated problems or delays and actions or plans to resolve

Nothing to Report.

Changes that had a significant impact on expenditures

Nothing to Report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to Report.

PRODUCTS

Publications, conference papers, and presentations

While unrelated to the aims described above, this grant has allowed me to reflect on my clinical experiences and publish a piece in the Journal of Clinical Oncology's Art of Oncology section regarding the use of non-approved cancer therapies through "compassionate use" protocols (attached in Appendix).

Website(s) or other Internet site(s)

Nothing to Report.

Technologies or techniques

Nothing to Report.

Inventions, patent applications, and/or licenses

Nothing to Report.

Other Products

Nothing to Report.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: David Einstein, MD
Project Role: PI
Researcher Identifier: 0000-0001-9163-3281
Nearest person month worked: 6
Contribution to Project: Organizing clinical data, generating research questions, analyzing SCNA and ctDNA data
Funding Support: this award

Name: Steven Balk, MD/PhD
Project Role: Mentor
Researcher Identifier: 0000-0002-4546-7371
Nearest person month worked: 1
Contribution to Project: Supervising research questions and data analysis
Funding Support: NIH R01, P01, P50 grants; DoD Impact Award W81XWH-16-1-0431 and Idea Development Award PC170715

Name: Adam Sowalsky, PhD
Project Role: Collaborator
Researcher Identifier: 0000-0003-2760-1853
Nearest person month worked: 2
Contribution to Project: Conducting WES and ctDNA sequencing
Funding Support: DoD W81XWH1610433 and W81XWH1510710

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report.

What other organizations were involved as partners?

Nothing to Report.

APPENDIX

ART OF ONCOLOGY

Compassion and Compassionate Use

David J. Einstein, Beth Israel Deaconess Medical Center, Boston, MA.

DOI: 10.1200/JCO.18.00205 *Journal of Clinical Oncology*

PMID: 30156981

The e-mail came down to this: “This patient is running out of options, and he and his family are starting to think ‘outside the box.’” Two months prior, the sender was my cofellow. Now, after a blissful vacation month entirely unplugged from oncology, he and I were junior attending physicians, launched into the dual frontlines of inpatient and outpatient oncology. He was staffing the consult service, I, the inpatient oncology service, both of us simultaneously trying to keep our back-burner outpatient clinics and research projects from boiling over. He was letting me know about my newest admission, a patient with advanced choriocarcinoma, which was ostensibly within my area of expertise of genitourinary cancers.

During years of residency training, we had learned to eyeball patients and differentiate sick from not sick—or really, those who might be imminently dying from those who were okay for now. As oncology fellows, we recognized a new group, the patients who seemed to be imminently dying on paper but in person looked amazingly normal. The patient referred to in the e-mail was such a patient, an energetic engineer in his fifties, with a more-than-slight Boston accent despite his years in California and Texas, and plenty of Red Sox gear to match. On paper, he was in dire shape, with treatment-resistant advanced cancer and increasing toxicities of treatment.

The patient and his family had taken on his disease with the battle mentality that works for some and makes others cringe. After multiple rounds of chemotherapy, resections, and radiation treatments failed to cure his disease, he moved on to the high-dose chemotherapy with autologous stem cell rescue that is the standard of care in this setting. If some chemotherapy is good, more is better. However, he eventually exhausted his supply of stem cells, still without having achieved remission. Time to think outside the box. Turning to his identical twin brother as a source of new stem cells, the patient proceeded to a last-ditch effort at high-dose chemotherapy, this time with syngeneic

transplantation. It worked—for approximately 3 months. Despite the most intensive efforts, his disease kept bouncing back. At this point, it was clear that chemotherapy would only transiently suppress his cancer but not cure it. Meanwhile, his treatments left him with hearing loss severe enough to require hearing aids, poor kidney function, foot numbness, and bone marrow that even on imaging looked exhausted, plus prolonged cytopenias to prove it.

What could be done? This patient and his family had crossed the country in search of an outside-the-box treatment that might offer him the benefit that standard treatments had not. We are used to patients looking online for new diets and complementary medicines; we understand that this is a way to seek an active part in their care when they otherwise feel like passive recipients. The patient and his family had gone further, identifying a clinical trial available at our hospital that they desperately wanted to join. But the trial was in a different tumor type, and moreover, he would not be a candidate for any kind of trial, given his accumulated treatment-related toxicities. Undaunted, his sister found an online poster from our peer institution across the street describing a novel compound used to treat a patient with his disease, with a resulting (and seemingly miraculous) remission. Again, we are accustomed to the patient who brings in the latest online research to an appointment. Sometimes, it is not scientifically valid, sometimes it is valid but not applicable to the situation, and occasionally, it is both valid and applicable and actually does result in a treatment change. As I scrutinized the information the patient's sister provided, it actually seemed both possibly valid and applicable. I could find no obvious toxicity that precluded use of this drug. And yet, it was early in testing, far from full approval. "What about compassionate use?" the family asked.

"Compassionate use" was a term I had only heard spoken of dismissively. We oncologists prided ourselves on always being compassionate, whether we were administering therapies or stopping them. Treating outside of standard of care without the structure of a research study is dangerous territory. But this concept has a popular appeal: who wouldn't want to try anything that could help in a desperate situation? Recently, proponents of so-called right-to-try legislation claimed that Food and Drug Administration (FDA) regulation was interfering with patients' access to experimental therapies outside of trials; the FDA replied that they approved 99% of expanded-access requests. Indeed, compared with the regulatory hurdles I faced from my own institution, I discovered that the FDA was the smallest barrier of all.

As I learned, creating a single-patient Investigational New Drug application for compassionate use was the ultimate in personalized medicine—a clinical trial created for one person. In fact, it was a desperate hope thrown into the slow-moving and opaque gears of our clinical research regulation, only to be chewed into a million tracked-changes documents and flurries of e-mails. I was rapidly assembling a trial protocol to use highly experimental therapy in a patient whose disease was bound to cause suffocation or bleeding at any moment. It was a terrifying, anxiety-provoking situation for physician, patient, and family alike. I felt caught between, on the one side, an eager and aggressive patient who wanted to sign anything, cover any cost, just to have a shot at a miracle, and on the other side, a process purposefully built to be as cautious as possible. I caught myself erupting in frustration, unfairly lashing out at the secretary who e-mailed me with institutional review board (IRB) edits on an informed consent form that the patient would never read.

Just as we were getting ready to sign this consent form, the patient's disease was making itself more plainly visible in the form of a bleeding superficial metastasis. Now, another regulatory catastrophe: between the bleeding lesion and the need for radiation, he was ineligible for the "trial" because of the strictly worded inclusion criteria used in the original protocol. Time for yet another urgent amendment, pleading for the IRB not to hold me to the rules that I myself had provided.

When, after months of work, the patient received a single dose of the study treatment, we all felt like we had climbed Everest. All along the way, I tried to frame this as a victory in and of itself, hoping he would die feeling he had tried everything. But he, of course, wanted to live, through sheer will and perseverance. And, I'll admit, a small portion of me wanted to prove my doubts wrong, to be the junior attending daring enough to try something audacious and land a huge, unconventional win.

It was not to be. After many tense clinic appointments and late-night calls during my drive home, our last conversations unfortunately occurred in the intensive care unit. With the rush of the high-flow nasal cannula as background white noise, I told the patient that his disease was not even responding to the chemotherapy I had tried a week ago in an ultra-last-ditch effort to get him back to the study drug. I could not pull him back from the edge of that ultimate cliff. Although I had tried hard to have the appropriate discussions with the patient and his family on many occasions—trying to refocus their hopes on spending good time together, freedom from pain and breathlessness, and another day outside the infusion area and the oncology ward—I

knew they felt I had failed them, that maybe pushing just a little harder would have made the difference. One morning, I awoke to an e-mail from the intensive care unit resident letting me know that the patient had died that night. Thankfully, we had agreed that aggressive interventions in his dying moments would be even less effective than our chemotherapies, and instead, he had received much more effective supportive care.

I let out a breath of simultaneous relief and regret. I knew this moment had been coming, of course. I had disagreed with my patient and his family about how to prepare for it, but my team and I had tried to make the patient and his family feel that every effort had been made on his behalf. I felt the failure not in his inevitable death, but in never arriving at a moment of shared celebration for our combined efforts, when we could say together, even in that intensive care unit room, "It was all worth it." And when I went to contact his wife and sister, I never heard from them again. I certainly did not fault them for not returning my calls or e-mails during the midst of intense grief. Yet, I never heard whether they felt that our compassionate use of a single dose of experimental treatment had helped or hurt. He went from being my all-consuming focus to a vanished spirit, and I had to wonder whether the family's silence gave me my answer.

The recent right-to-try legislation cleared the US Senate last year and the House of Representatives in March, along mostly partisan lines. Opponents, including representatives from the American Society for Clinical Oncology as well as the American Cancer Society, pointed out the value of FDA oversight and the existing pathway for access to experimental therapies outside of clinical trials. Regardless of the fact that this legislation does not affect the institutional barriers I encountered, I do wonder whether it will inspire a growing number of desperate patients to exercise their right to try.

How should oncologists respond? Except in the cases of a drug that seems to have particularly exciting preclinical justification or early clinical data, I suspect most will advise their patients with advanced cancer to stick to more trodden territory: to try accepted anticancer therapies when they are judged to be reasonable and to switch to supportive care alone when the risk seems to outweigh the benefit (individualized to patient preferences). "As we continue to chase progress in cancer," Wachter[1] recently wrote, "let's be sure that we don't rob dying patients of a smaller, more subtle miracle: a death with dignity and grace, relatively free from pain and discomfort." But there will always be the patients like mine, unsatisfied to do nothing, as much as we try to reinforce that intensive supportive care is far from nothing. I recall the words of another

oncologist who had seen the patient for a second opinion: “These patients [with refractory testicular cancer] die with the chemo going in.” Chemotherapy or, perhaps increasingly, experimental drugs.

If we are to routinely offer such patients access to experimental drugs outside of clinical trials, then we will need to address several issues in advance. First, federal and local regulatory burdens will have to be substantially lower. We will need to simplify the process of writing a protocol, obtaining IRB approval, and amending the protocol as clinical circumstances evolve. (As a word of advice, oncologists embarking on such a process should be careful about recycling protocols written for standard trials; in particular, because these protocols are designed around a specific patient, the inclusion and exclusion criteria should be minimal.) Second, directors of clinical trial programs will have to decide whether their operations are capable of diverting resources from standard trials to these urgent and unfunded projects. Finally, and most importantly, individual clinicians will have to develop strong end-of-life communication skills to help frame a shared decision-making process properly, and they may even have to make especially difficult decisions about when to exercise a right to deny such requests. I hope that all of my patients will feel that I have treated them compassionately and with the tools best suited to their situation—whether or not this includes compassionate use of unproven therapies. And I hope that my patient did indeed feel that we left no stone unturned and that he felt some small satisfaction in this.

Supported by Congressionally Directed Medical Research Programs (CDMRP) Prostate Cancer Research Program (PCRP) Physician Research Award #W81XWH-17-1-0350.