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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. Furthermore, identification of phenotypic subtypes amenable to strategies such as immunotherapy could enhance personalized treatment. During this period, we continued to expand our cohort of non-indolent active surveillance (AS) patients in order to provide a comparitor for the relatively quiet copy-number landscape we previously identified in indolent AS patients. We also investigated a novel phenotype of immunogenic localized PCa characterized by an apparent anti-tumor immune response. Finally, we investigated driver mutations in localized PCa and examined how these were affected by metastatic spread and treatment resistance.

15. SUBJECT TERMS
Prostate cancer, active surveillance, biomarkers

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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. Thus, one goal of this project was 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 hypothesized that truly indolent Gp3/Gs6 tumors were a molecularly distinct subset and will have a relatively silent SCNA landscape, while potentially aggressive Gp3 tumors would have extensive SCNA including losses in established tumor suppressor genes. Furthermore, in an extension of our efforts to biologically subset different types of localized PCa, we investigated a novel immunogenic subset of localized PCa, and we characterized driver alterations in localized PCa and the effect of metastatic spread and treatment resistance on response to targeted treatment of these driver alterations.

2. KEYWORDS

Prostate cancer, active surveillance, biomarkers

3. ACCOMPLISHMENTS

What were the major goals of the project?

Research-Specific Tasks

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.

Training-Specific Tasks

Major Task 1: Training and educational development in prostate cancer research

What was accomplished under these goals?

Research-Specific Tasks

Aim 1:

Subtask 1: Identify appropriate tissue samples (ongoing, 75% completed)

We are evaluating SCNA as a prognostic biomarker regarding probability of clinical progression on active surveillance. We previously identified cases of patients who remained clinically indolent with long-term follow-up on active surveillance as well as a few cases of patients who were upgraded from low-risk initial disease to higher-risk disease subsequently (clinically non-indolent), requiring primary therapy. We did not include any patients in the non-indolent cohort who were upgraded on first re-biopsy, since this is typically thought to reflect sampling error rather than true disease progression. Thus, all non-indolent patients in this study were not found to have higher-risk disease until after several years' follow-up. From each case, we identified biopsies taken at baseline; in patients who had no malignancy on initial biopsies, we selected the first biopsy that showed malignancy.

In order to expand the cohort, I identified BIDMC active surveillance patients enrolled in the PASS study (NCT00756665) who ultimately required primary therapy, providing a comparison cohort for the established indolent cases.

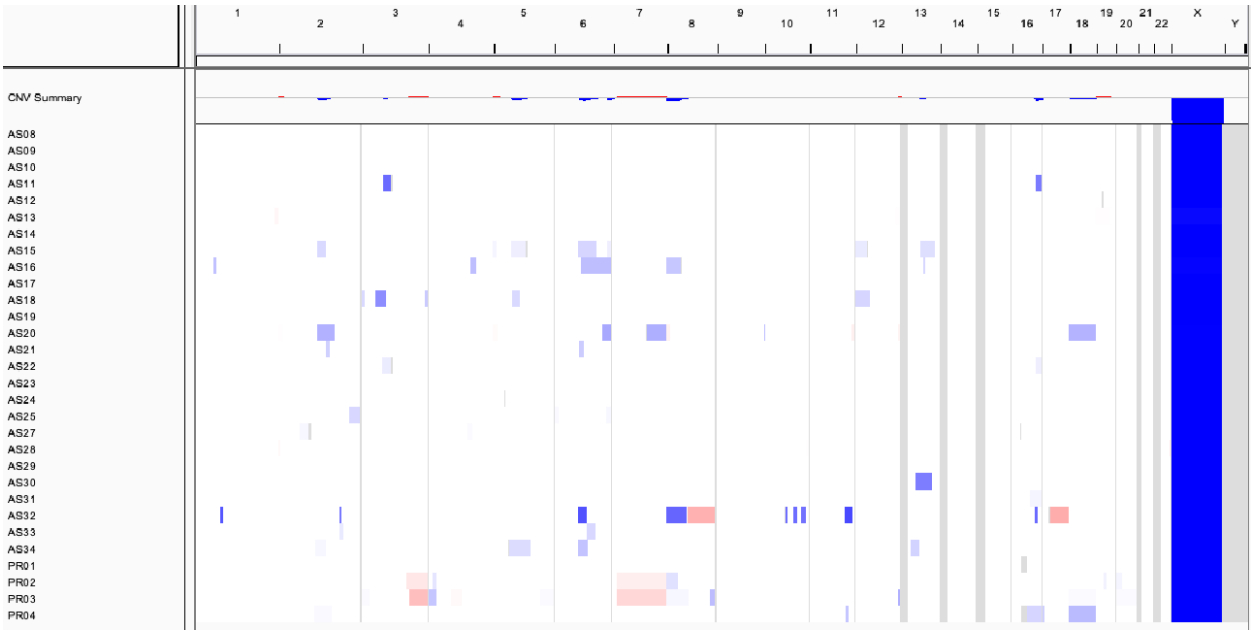
Subtask 2: Microdissect (ongoing, 75% completed)

After selecting appropriate blocks from each case, we performed punch biopsies from the areas most involved by tumor for subsequent cases. We also obtained benign tissue for comparison. The same procedure is planned for the newly identified samples described above, but we have been limited by the availability of pathology resources.

Subtasks 3 and 4: Evaluate SCNA from tumor and from normal prostate tissue and perform cross-platform analyses (ongoing, 75% completed)

We previously extracted DNA from these samples and performed ultra-low-pass whole-genome sequencing (ULP-WGS).

Figure 1: ULP-WGS from Clinically Indolent and Non-Indolent Prostate Cancer Managed with Active Surveillance

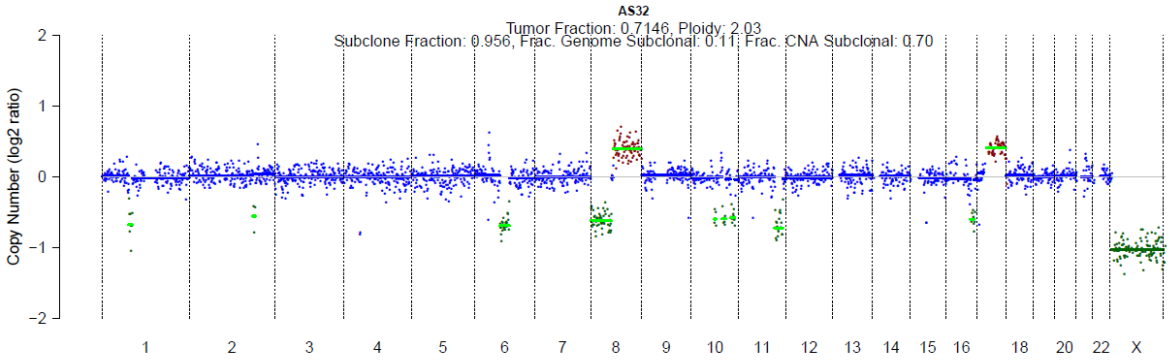


As previously described (Figure 1), the SCNA landscape was quiet with few CN alterations, especially among patients with clinically indolent prostate cancer. Among patients who had clinically non-indolent prostate cancer, one case (PRO2/3) demonstrated gain in chromosomes 3 and 7. However, PRO1 and PRO4 had no significant SCNAs despite experiencing clinical progression.

Six of the clinically indolent cases had losses in 6q. Loss of heterozygosity at 6q16-22 is frequently detected in both aggressive and non-aggressive prostate cancer, leading to a hypothesis that tumor suppressor genes in this area were likely to play a role in initiation of cancer but not in progression (Lu & Hano, Prostate Cancer & Prostatic Disease 2008).

Interestingly, in one patient from the clinically indolent group, copy number alterations were seen in chromosome 6 (q loss) as above but also chromosomes 8 (p loss and q gain), 11 (loss), and 17 (gain) (Figure 2). Although ULP-WGS cannot distinguish individual genes, these locations encompassed NKX3.1 loss (chromosome 8p), MYC gain (chromosome 8q), ATM loss (chromosome 11q). Moreover, this patient had a possible single-copy PTEN loss (10q). Overall ploidy was 2.03. This contrasts with other literature suggesting that early alterations in ATM and MYC subclones predict for occult metastatic disease at time of radical prostatectomy (Espiritu SMG et al., Cell 2018; Fraser M et al., Nature 2017; Sowalsky AG et al, Clin Cancer Res 2017). We will continue to follow all of these patients clinically to ensure they do indeed remain clinically indolent.

Figure 2: Genomic instability in one patient with clinically indolent prostate cancer



In another patient with clinically indolent disease (AS16), there was loss of 8p, but this was also seen in 2 cases of non-indolent disease.

SCNA will be analyzed in the newly identified samples described above.

Subtask 5: Begin expansion to larger cohort if indicated (ongoing, 50% complete)

We have decided to further investigate the SCNA landscape of clinically non-indolent cases in order to see whether a quiet landscape at diagnosis is predictive of subsequent outcomes. As above, I identified an additional cohort of patients to expand our non-indolent cases. I have assembled a list of appropriate blocks and have confirmed clinical data. We are identifying available pathologists to help complete this work.

Sub-Aim 1b: Characterize the immune tumor microenvironment of Gp3 versus Gp4-associated Gp3 in patients on AS (will not be performed)

Our preliminary findings from our immunogenic prostate cancer project (described further below) revealed that it was difficult to capture immune TME in core needle biopsies given tissue heterogeneity in prostatectomy samples.

Aim 2:

We planned to first study ctDNA as a potential biomarker in patients with intermediate-/high-risk localized prostate cancer with the rationale that if it could not be reliably detected or correlated with outcome in these patients, that it would also likely be limited as a biomarker in earlier disease. We published our results from this study (Hennigan ST et al., JCO Precision Oncology 2019).

Major Task 1: Identify tumors overexpressing ERG (completed)

As reported previously, we performed IHC to establish ERG status of tumors, and we selected concordantly positive or negative foci (supplement table 5 in manuscript cited above).

Major Task 2: Identify TMPRSS2:ERG breakpoint (completed)

As reported previously, we successfully microdissected tumor foci, extracted DNA, and constructed a genomic DNA library. We performed whole-genome sequencing to identify the breakpoint and used these results to generate breakpoint-specific primers.

Major Task 3: Quantification of breakpoint in ctDNA (completed)

As reported previously, we extracted DNA from plasma samples and attempted to amplify the TMPRSS2:ERG fusion with primers generated above. In one of two ERG-positive cases for which WGS was performed, we successfully read through the TMPRSS2:ERG breakpoint (Supplement Figure 3a-c in cited manuscript). However, even a nested PCR approach failed to amplify the fragment of DNA containing the breakpoint from plasma (Supplement Figure 3d-e in cited manuscript).

Major Task 4: Perform sequencing of RP specimens and look for identified tumor mutations in ctDNA (completed)

In plasma collected pre-RP from 112 patients, we did not find any SCNAs via ULP-WGS. This contrasted with results from patients with metastatic disease, where 4/7 patients had detectable ctDNA by ULP-WGS (see cited manuscript for full details).

We then tried a personalized ultradeep sequencing approach. This method involved laser capture microdissection of multiple geographically and phenotypically distinct tumor foci from prostatectomy specimens in order to identify truncal mutations. Once identified, these would serve as targets for ultradeep sequencing of matching ctDNA. After confirming this method's sensitivity with spike-in experiments, we attempted this approach in nine patients with localized intermediate-/high-risk disease undergoing prostatectomy. However, we were unable to detect any ctDNA in plasma from any of these patients before or after RP, even in a patient who subsequently experienced biochemical recurrence (and therefore, by definition, had micrometastatic disease present at the time of surgery).

Major Task 5: Expand analysis to lower-risk tumors (will not be performed)

Based on these findings and according to criteria set forward in the Statement of Work, we previously concluded that ctDNA was unlikely to be present in sufficient amounts in lower-risk tumors to be useful as a biomarker in this setting.

Training-Specific Tasks

Subtask 1: Attend and present at an international scientific meeting (completed and ongoing)

I have presented a variety of abstracts at scientific meetings over the past 4 years (see Products below).

Subtask 2: Organize and present research at weekly GU tumor board (completed and ongoing)

I continue to coordinate our weekly multidisciplinary GU oncology tumor board, assembling lists of patients to be discussed, distributing this to radiologists and pathologists, and presenting new literature in the field for discussion. During COVID-19, I completely shifted our tumor board to an online format and worked together with a programming team to develop an online case submission system.

Subtask 3: Complete courses in biostatistics, biomarker development, and computational biology (completed biostatistics course; not yet initiated biomarker development or computational biology courses)

I obtained a certificate in biostatistics from the Harvard Catalyst program.

Subtask 4: Publish results in a peer-reviewed journal (completed)

I was part of a team that previously published our investigation of ctDNA in localized prostate cancer in the Journal of Precision Oncology. In addition, I published describing similar ctDNA work in a patient with esophageal cancer (Einstein DJ et al., JCO Precision Oncol 2020).

I was senior author on a publication describing a novel immunogenic subset of prostate cancer (Appendix 1; Calagua C et al., Clin Canc Res 2021). I was first author on a publication describing an in-depth genomic and functional analysis of tumor evolution using rapid autopsy materials (Appendix 2; Einstein DJ et al., JCO Precision Oncol 2021).

Subtask 5: Develop, write, obtain regulatory approval, and activate a trial of polo-like kinase inhibitor in patients with metastatic castration-resistant prostate cancer and early resistance to Abiraterone (completed)

I am overall PI of NCT03414034, which has accrued over 50 patients to date to test the combination of onvansertib (PLK1i) and abiraterone.

Subtask 6: Develop, write, obtain regulatory approval, and activate a trial of PD-1 inhibitor in patients with high-risk biochemically recurrent prostate cancer (completed)

I am overall PI of NCT 03637543, which has accrued over 25 patients to date to test the use of nivolumab in high-risk biochemically recurrent prostate cancer.

Subtask 7: Collect tumor tissue and blood samples from patients undergoing radiation plus androgen deprivation therapy for localized prostate cancer, evaluate for presence of T cells capable of recognizing tumor neoantigens identified in biopsy samples (stopped, 25% completed, will not be continued)

We completed tumor and blood sample collection from 12 patients. We performed WES from four cases, predicted neoantigens, and generated peptides based on these neoantigens. We performed exploratory experiments stimulating PBMCs with these peptides. However, preliminary evidence did not support expanding this analysis.

Subtask 8: Establish a rapid autopsy program for patients deceased of prostate and other genitourinary cancers, develop a tissue bank, perform IHC studies, establish xenografts and organoids from autopsy tissue (completed and ongoing)

I have set up a successful program for performing rapid autopsies. This program is a component of the Pathology Core in the recently submitted Prostate SPORE application and is intended to generate collaboration between DF/HCC scientists and clinicians, including investigators at both Dana-Farber Cancer Institute (DFCI) and Beth Israel Deaconess Medical Center (BIDMC). Since its inception and through the incredible generosity of our patients and their families, we have performed >10 rapid autopsies, predominantly in patients deceased of prostate cancer. At the time of autopsy, which is typically within three hours of death, we collect material from approximately 10-20 sites including bone, nodal, and visceral metastases. We collect both fixed formalin paraffin embedded tissue and matched frozen tissue. We have also collected fresh tissue for viable freezing and have created a patient-derived xenograft (PDX) from one case. All of these patients had been treated with standard hormone therapy, including androgen signaling inhibitors, and several have additional archival specimens available from time of diagnosis—in the localized or metastatic setting—and before and/or after subsequent therapies. In some cases, we have matched pre/post-treatment biopsies, and some of these patients have also been treated with investigational agents and/or specialized therapies such as immune checkpoint inhibitors for microsatellite-high disease and poly(ADP)-ribose polymerase inhibitors for tumors with deficiencies in homologous recombination repair. This program is IRB reviewed and involves patient research consent for obtaining tissue for research purposes, including establishment of cell lines, organoids, and PDXs. We recently published one paper involving rapid autopsy tissue. Another is under review.

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. During the course of this project, I have developed skills that are essential for this career path. I have worked closely with colleagues in pathology to develop a better understanding of tissue preparation including how to isolate small amounts of tumor from core biopsies and assemble a tissue micro-array. I have worked with Drs. Balk and Sowalsky to analyze and interpret sequencing data. Finally, I have worked with regulatory specialists to better understand regulatory issues surrounding tissue banking studies. I have leveraged all of these skills for other projects, especially the rapid-autopsy protocol.

The salary support offered by this award has also protected time to pursue a number of projects ranging from exploratory pre-clinical work to phase II clinical trials.

As above, I have set up a rapid-autopsy protocol as above. 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.

I have had the opportunity to design and run 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 (mCRPC). The other involves the PD-1 inhibitor nivolumab for patients with high-risk biochemically recurrent prostate cancer. Both trials are currently open and accruing patients, and both have led to successful grant proposals for correlative studies. I have also successfully obtained funding for a third phase 2 clinical trial involving neratinib in a biomarker-selected population of patients with mCRPC, which was activated briefly before the sponsor unfortunately withdrew funding.

Finally, I have used some of my protected time to contribute to several research manuscripts and reviews, as outlined below. I also wrote the newest version of the Genitourinary Oncology section of the ASCO Self-Evaluation Program used to train oncology fellows nationally and internationally, and I added several updates after initial publication.

How were the results disseminated to communities of interest?

I have published multiple manuscripts as detailed below. I created a Twitter "tweetorial" outlining our findings from immunogenic prostate cancer. I presented these findings at a Prostate Cancer Foundation Tumor Microenvironment Working Group meeting, and I have submitted an abstract to the Multi-Institutional Prostate SPORE retreat.

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

Nothing to report

IMPACT

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

Our findings regarding immunogenic prostate cancer (Calagua C et al., Clin Canc Res 2021) have generated new interest in targeting immunotherapies to a novel disease space and examination of biomarkers for identifying these patients. We are generating data to support new trials of neoadjuvant approaches prior to surgery.

Our findings regarding targetable driver alterations in primary prostate cancer that remain targetable after acquisition of additional genomic events (Einstein DJ et al., JCO Precision Oncology 2021) may influence timing of genomic testing in prostate cancer and understanding of tumor evolution.

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?

The provided funding allowed me to support time spent on patient outreach and community engagement, including becoming a medical advisor to the Boston Prostate Cancer Support Group and the Massachusetts Prostate Cancer Coalition.

CHANGES/PROBLEMS

Changes in approach and reasons for change

Aim 1:

No changes other than including more cases as noted above. We hope to have available pathology resources in the future for continuing tumor scraping to complete this Aim.

Aim 2:

Given the difficulty detecting ctDNA even in more aggressive localized tumors, even one with biochemical recurrence, we decided that this approach is likely to be limited as a biomarker in more indolent early-stage disease, where ctDNA is even less likely to be present.

We will continue to follow patients analyzed in our previous cohort to see if they develop metastatic recurrence. If so, we will try to obtain biopsy material. This would allow us to verify that our personalized primers were targeting the correct clone, the one ultimately responsible for metastatic recurrence.

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

Publications

1. **Einstein DJ,** Desanto-Madeya S, Gregas M, Lynch J, McDermott DF, and Buss MK. Improving end of life care: palliative care embedded in an oncology clinic specializing in targeted and immune-based therapies. J Oncol Pract. 2017; 13(9):e729-e737. [PMID: 28562197]
2. Russo JW, Liu X, Ye H, Calagua C, Chen S, Voznesensky O, Condulis J, Ma F, Taplin ME, **Einstein DJ,** Balk SP, Chen S. Phosphorylation of androgen receptor serine 81 is associated with its reactivation in castration-resistant prostate cancer. Cancer Letters. 2018; 438:97-104. [PMID: 30217568]
3. **Einstein DJ**, Patil D, Chipman J, Regan MM, Davis K, Crociani CM, Wagner AA, Sanda M, Chang P. Expanded Prostate Cancer Index Composite-26 (EPIC-26) online: validation of an internet-based instrument for assessment of health-related quality of life after treatment for localized prostate cancer. Urology. 2019;127:53-60. [PMID: 30790648]
4. Henningan ST, Trostel SY, Terrigino NT, Voznesensky OS, Schaefer RJ, Whitlock NC, Wilkinson S, Carrabba NV, Atway R, Shema S, Lake R, Sweet AR, **Einstein DJ**, Karzai F, Gulley JL, Chang P, Bubley GJ, Balk SP, Ye H, Sowalsky AG. Low abundance of circulating tumor DNA in localized prostate cancer. JCO Precision Oncology 2019;3. [PMID: 31528835]
5. **Einstein DJ**, Liang N, Malhotra M, Aleshin A, Moshkevich S, Billings PR, Pectasides E. Assessment of Molecular Remission in Oligometastatic Esophageal Cancer with a Personalized Circulating Tumor DNA Assay. JCO Precision Oncology 2020;4:239-243. [DOI: 10.1200/PO.19.00339]
6. Awasthi S, Berglund AE, Abraham-Miranda J, Rounbehler RJ, Kensler KH, Serna AN, Vidal AC, You S, Freeman MR, Davicioni E, Liu Y, Karnes JR, Klein EA, Den RB, Trock BJ, Campbell JD, **Einstein DJ**, Gupta R, Balk SP, Lal P, Park JY, Cleveland JL, Rebbeck TR, Freedland SJ, Yamoah K. Comparative genomics reveals distinct immune-oncologic pathways in African American men with prostate cancer. Clin Cancer Res. 2020;27(1):320-329. [PMID: 33037017]
7. Patell R, **Einstein DJ**, Miller E, Dodge L, Halleck J, Buss M. Patient Perceptions of Treatment Benefit and Toxicity in Advanced Cancer: a prospective cross-sectional study. J Oncol Pract 2021;17(2):e119-e129. [PMID: 33444075]
8. Morel K, Sheahan A, Burkhart D, Baca S, Labbe D, Canadas I, Roehle K, Heckler, Calagua C, Ye H, Pantelidou C, Galbo P, Panja S, Mitrofanova A, Wilkinson S, Whitlock N, Trostel S, Hamid A, Kibel A, Barbie D, Choudhury A, Pomerantz M, Sweeney C, **Einstein D**, Shapiro G, Dougan S, Sowalsky A, Freedman M, Balk S, Loda M, Olson B, Ellis L. EZH2 Inhibition Activates a dsRNA-STING-Interferon Stress Mechanism that Potentiates Response to PD-1 Checkpoint Blockade in Prostate Cancer. Nature Cancer 2020;2:444-456. [PMID: 33899001]
9. McKay RR, Xie W, Ye H, Fennessy FM, Zhang Z, Lis R, Calagua C, Rathkopf D, Laudone VP, Bubley GJ, **Einstein DJ**, Chang PK, Wagner AA, Parsons JK, Preston MA, Kilbridge K, Chang SL, Choudhury AD, Pomerantz MM, Trinh QD, Kibel AS, Taplin ME. Results of a randomized phase II trial of intense androgen deprivation therapy prior to radical prostatectomy in men with high-risk localized prostate cancer. J Urol 2021. *Online ahead of print.* [PMID: 33683939]

10. Calagua C, Ficial M, Jansen CS, Hirz T, Balzo L, Wilkinson S, Lake R, Ku AT, Voznesensky OS, Sykes DB, Saylor PJ, Ye H, Signoretti S, Kissick HT, Sowalsky AG, Balk SP, **Einstein DJ**. A Subset of Localized Prostate Cancer Displays an Immunogenic Phenotype Associated with Losses of Key Tumor Suppressor Genes. Clin Cancer Res 2021; 27(17):4836-4847. [PMID: 34168052]
11. Cole SM, Sarangi S, **Einstein DJ,** McMasters M, Alterman R, Bruce J, Hertan L, Shih HA, Wong ET. Parkinsonism reversed from treatment of pineal non-germinomatous germ cell tumor. Surg Neurol Int 2021;12(237):1. [PMID: 34221568]
12. **Einstein DJ***, Arai S*, Calagua C, Xie F, Voznesensky L, Capaldo B, Luffman C, Hecht JL, Balk SP, Sowalsky AG, Russo J. Metastatic castration-resistant prostate cancer remains dependent on oncogenic drivers found in primary tumors. JCO Precision Oncology 2021;5:1514-1522 [DOI: 10.1200/PO.21.00059].

1. **Einstein DJ.** Knot. Ann Intern Med. 2012;157(5):381-2. [PMID: 22944877]
2. **Einstein DJ,** Trowbridge RL, and Rencic J. A problematic palsy: an exercise in clinical reasoning. J Gen Intern Med. 2015;30(7):1029-33. [PMID: 25743429]
3. **Einstein DJ**, Ladin K, and Mathew P. The ethical imperative of healthy paternalism in advance- directive discussions at the end of life. JAMA Oncology. 2016;2(4):429-30. [PMID: 26914544]
4. **Einstein DJ** and McDermott DF. Combined blockade of vascular endothelial growth factor and programmed death 1 pathways in advanced kidney cancer. Clinical advances in hematology & oncology. Clin Adv Hematol Oncol 2017;15(6)478-488. [PMID: 28749908]
5. **Einstein DJ.** Compassion and Compassionate Use. J Clin Oncol. 2018; 36(29):2969-2971. [PMID: 30156981]
6. **Einstein DJ** and Sonpavde G. Optimizing management of cisplatin-ineligible bladder cancer patients. Current Treatment Options in Oncology. 2019;20(2):12. [PMID: 30741358]
7. **Einstein DJ**, Arai S, and Balk SP. Targeting the Androgen Receptor and Overcoming Resistance in Prostate Cancer. Current Opinion in Oncology. 2019;31(3):175-182. [PMID: 30893145]
8. **Einstein DJ** and Garnick MB. Genitourinary Cancers chapter of American Society of Clinical Oncology Self-Evaluation Program, 7th Edition. pp. 403-476.
9. Bilusic M, **Einstein DJ**, Karzai FH, Dahut WL, Gulley JL, Aragon-Ching JB, Madan RA. The Potential Role for Immunotherapy in Biochemically Recurrent Prostate Cancer. Urol Clin North Am 2020;47(4):457-467. [PMID: 33008496]
10. Halbert B and **Einstein DJ**. Hot or not: tumor mutational burden (TMB) as a biomarker of immunotherapy response in genitourinary cancers. Urology. 2020. *In press.*
11. Santopietro AL, **Einstein DJ**, Bellmunt J. Advances in the management of urothelial carcinoma: is immunotherapy the answer? Expert Opin on Pharmacother. 2021. *Online ahead of print*. [PMID: 33905290]

1. Garnick MB and **Einstein DJ.** Editorial comment. Urology. 2019;131:182-183. [PMID: 31451158]
2. Garnick MB and **Einstein DJ.** Editorial comment. Urology. 2020;145:118-119. [PMID: 33167168]

Presentations

1. **Einstein DJ**, Wei XX, Werner L, Ye H, Calagua C, Bubley G, Balk SP. A phase II study of nivolumab in patients with high-risk biochemically recurrent (BCR) prostate cancer (PCa). ASCO Genitourinary Cancers Symposium 2019, San Francisco, CA.
2. **Einstein DJ,** Avigan DE, Bhasin MK, Freeman GJ, Mahoney KM, Stroopinsky D, Rosenblatt J, Wei XX, Werner L, Wu CJ, Ye H, Balk SP. Identifying and Targeting Immunogenic Prostate Cancer at High Risk for Lethal Metastatic Progression. 12th Annual Multi-Institutional Prostate Cancer Program Retreat. Ft. Lauderdale, FL.
3. **Einstein DJ,** Choudhury AD, Saylor PJ, Werner L, Erlander MG, Ridinger M, Bubley G. A phase II study of onvansertib (PCM-075) in combination with abiraterone and prednisone in patients with metastatic castration-resistant prostate cancer. ASCO Genitourinary Cancers Symposium 2019, San Francisco, CA.
4. **Einstein DJ,** Choudhury AD, Saylor PJ, Werner L, Erlander MG, Ridinger M, Bubley G. A phase II study of onvansertib (PCM-075) in combination with abiraterone and prednisone in patients with metastatic castration-resistant prostate cancer. AACR Annual Meeting 2019, Atlanta, GA.
5. **Einstein DJ,** Wei XX**,** Avigan DE, Bhasin MK, Freeman GJ, Mahoney KM, Stroopinsky D, Rosenblatt J, Werner L, Wu CJ, Ye H, Balk SP. Identifying and targeting immunogenic prostate cancer at high risk for lethal metastatic progression. 26th Annual Prostate Cancer Foundation Scientific Retreat 2019. Carlsbad, CA.
6. Russo JW, Gao X, **Einstein DJ**, Bubley GJ, Balk SP. Aberrant HER2 signaling is a therapeutic target in a subset of castration-resistant prostate cancer. AACR Annual Meeting 2019, Atlanta GA.
7. Patell R, **Einstein DJ**, Halleck J, Buss MK. Patient perceptions of treatment benefit in advanced cancer. ASCO Supportive Care in Oncology Symposium 2019. San Francisco, CA.
8. Shaw K, Calagua C, Russo J, **Einstein DJ**, Balk S, Ye H. Tumor PD-L1 expression is detected in a significant subset of high-risk localized and metastatic prostate cancer but is rare in ductal subtype. United States and Canadian Academy of Pathology (USCAP) Annual Meeting 2019. Baltimore, MD.
9. Gupta S, Sonpavde G, Weight CJ, McGregor BA, Gupta S, Maughan BL, Wei XX, Gibb E, Thyagarajan B, **Einstein DJ**, Dechet CB, Lowrance WT, Murugan PJ, Kilbridge KL, Agarwal N, Davicioni E, Eckstein M, Mossanen M, Preston MA, Konety BR. Results from BLASST-1 (Bladder Cancer Signal Seeking Trial) of nivolumab, gemcitabine, and cisplatin in muscle-invasive bladder cancer (MIBC) undergoing cystectomy. ASCO Genitourinary Cancers Symposium 2020, San Francisco, CA. (selected oral abstract presented by Dr. Shilpa Gupta)
10. **Einstein DJ,** Choudhury AD, Saylor PJ, Werner L, Erlander MG, Ridinger M, Bubley G. A phase II study of onvansertib (PCM-075) in combination with abiraterone and prednisone in patients with metastatic castration-resistant prostate cancer. ASCO Genitourinary Cancers Symposium 2020, San Francisco, CA.
11. Kelly WK, Leiby B, **Einstein DJ**, Szmulewitz RZ, Sartor AO, Yang ES, Sonpavde G. Radium-223 and niraparib treatment in castrate-resistant prostate cancer patients with and without prior chemotherapy. ASCO Virtual Scientific Program 2020.
12. Choudhury AD, Xie W, Parikh M, Lee D, Kessler ER, **Einstein DJ,** Kochupurakkal B, Mouw KW, Van Allen EM, Doyle LA, D'Andrea AD, Taplin ME, Shapiro G. A phase II study of M6620 in combination with carboplatin compared with docetaxel in combination with carboplatin in metastatic castration-resistant prostate cancer. ASCO Virtual Scientific Program 2020.
13. McKay RR, Xie W, Fennessy FM, Zhang Z, Lis R, Rathkopf DE, Laudone VP, Bubley G, **Einstein DJ**, Chang P, Wagner A, Preston MA, Kilbridge KL, Chang SL, Choudhury AD,

Pomerantz M, Trinh QD, Kibel AD, Taplin ME. Results of a phase II trial of intense androgen deprivation therapy prior to radical prostatectomy in men with high-risk localized prostate cancer. ASCO Virtual Scientific Program 2020. (selected oral abstract presented by Dr. Rana McKay)

14. Patell R, **Einstein DJ**, Miller EJ, Halleck J, Dodge L, Buss MK. "Where did you read that?" External sources of information and patients' perceptions of prognostic goals. ASCO Virtual Scientific Program 2020.
15. Miller EJ, Patell R, **Einstein DJ**, Halleck J, Dodge L, Buss MK. Trends in patient misperception and decisional regret during treatment for advanced cancer: a prospective study. ASCO Virtual Scientific Program 2020.
16. Patterson JC, Croucher PJ, Ridinger M, **Einstein DJ,** Varkaris A, Balk SP, Bubley GJ, Erlander MG, Yaffe MB. The selective polo-like kinase (Plk1) inhibitor ovansertib and the antiandrogen abiraterone synergistically kill cancer cells through disruption of mitosis independently of androgen receptor signaling. AACR Virtual Annual Meeting 2021.
17. Halbert B, **Einstein DJ**, McDermott D, Andrianopoulos E, Gupta M, Seery V, Onimus K, Herman C, Wells A, Natarajan A, Veerapathran A, Bhatt RS. Successful generation of tumor-infiltrating lymphocyte (TIL) product from renal cell carcinoma tumors for adoptive cell therapy. SITC Annual Meeting 2021.

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 plus P20, Bridge Grant, and PCF Challenge Award noted below

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?

Completed and new funding are indicated below in italics.

Previous Support

P20-CA233255 (PI: Balk & Einstein) *04/01/19-08/31/20* *0.12 CM*
NIH/NCI *TDC:*
"Genomic Features of Immunogenic Prostate Cancer in African-American Patients"
Aim 1. Identify somatic genomic alterations associated with immunogenic PCa in AA patients
Aim 2. Identify germline genomic variants associated with immunogenic PCa in AA patients
Contact: Leah Gibbons;; gibbon@dfci.harvard.edu
31 Center Drive, Building 31
Bethesda, MD 20814

18CHAL09 (PI: Balk) *11/02/18-11/02/20* *1.2 CM*
Prostate Cancer Foundation *TDC:*
"Identifying and Targeting Immunogenic Prostate Cancer at High Risk for Lethal Metastatic Progression"
(I am PI of the phase 2 study that supports Aim 2, and I am PI of the translational protocol #17-048 that supports Aim 3.)
Aim 1. Identify genomic and microenvironmental features associated with immunosuppressive mechanisms in PD-L1-positive primary prostate cancer.
Aim 2. Determine predictive ability of PD-L1 expression in primary prostate cancer for response to nivolumab in men with biochemical relapse.
Aim 3. Examine antigen-specific T cell responses to mutated peptides expressed in prostate cancer cells, and whether these antigen-specific T cells are expanded by PD-1 blockade.
Contact: Howard R. Soule;; info@pcf.org
1250 Fourth Street
Santa Monica, CA 90401

W81XWH-17-1-0350 (PI: Einstein) *08/01/17-07/31/21* *6.0 CM*
Department of Defense *TDC:*
"Prognostic Biomarkers in Active Surveillance: Parsing Risk in Early-Stage Prostate Cancer"
Aim 1. Determine the somatic copy number alteration 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 prostate cancer.
Contact: CDMRP Help Desk;; help@eBRAP.org
1077 Patchel Street
Fort Detrick, MD 21702

Puma Biotechnology (PI: Einstein) *05/07/21-10/6/21*
"A Phase 2 Study of Neratinib for Men with Castration-Resistant Prostate Cancer and Evidence of Increased HER2 Signaling" (Investigator-initiated)
Contact: Deepa Lalla;; dlalla@pumabiotechnology.com
10880 Wilshire Blvd, Suite 2150
Los Angeles, CA 90024

Current Support

Bridge Grant (Co-PIs: Balk, Einstein, Yaffe) 03/01/19-02/28/22 0.6 CM
DF/HCC & Koch Institute TDC:
"Optimizing Plk1 Therapeutics for Clinical Translation"
Aim 1. Co-clinical trial to assess predictive biomarkers of synergy between Plk1 inhibitors and abiraterone.
Aim 2. Identification of mechanisms of synergistic cancer cell killing to expand the utility of this combination to other cancer types.
Contact: Deborah Goff;; deborah_goff@dfci.harvard.edu
450 Brookline Avenue, BP322A
Boston, MA 02215

Bristol-Myers Squibb (PI: Einstein) 06/11/18-Current
"A Phase 2 Study of Nivolumab in Patients with High-Risk Biochemically Recurrent Prostate Cancer." (Investigator-initiated)
Additional approved requests for funding from BMS and for discounted next-generation sequencing (through competitive application process) as a research collaboration with Foundation Medicine
Contact: Morgan Frazer;; morgan.frazer@bms.com
100 Binney Street
Cambridge, MA 02142

Cardiff Oncology (PI: Einstein) 06/07/18-Current
"A Phase 2 Study of Onvansertib (PCM-075) in Combination with Abiraterone and Prednisone in Subjects with Metastatic Castration-Resistant Prostate Cancer." (Joint Investigator/Industry-initiated)
Contact: Vicki Kelemen;; vkelemen@cardiffoncology.com

11055 Flintkote Avenue
San Diego, CA 92121

Young Investigator Award (PI: Einstein) *1.0 CM*
Prostate Cancer Foundation *TDC:*
"Identifying and Targeting Immunogenic Prostate Cancer"
This is aimed at characterizing the immune microenvironment and genomic signatures of immunogenic prostate cancer.
Contact: Howard R. Soule;; info@pcf.org
1250 Fourth Street
Santa Monica, CA 90401

What other organizations were involved as partners?

Multiplex panels for immunogenic prostate cancer research were performed by the Kissick Lab at Emory University (Atlanta, GA) and the Signoretti Lab at Dana-Farber Cancer Institute (Boston, MA). Computational analysis for this project and the rapid autopsy manuscript was provided by the Sowalsky Lab at the National Cancer Institute (Bethesda, MD)