

“N of 1” case reports in the era of whole-genome sequencing

A. Rose Brannon, Charles L. Sawyers

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Commentary

Prostate cancer has a range of clinical outcomes, from complete remission in response to treatment to death as a result of aggressive metastasis. Prognosis for individuals with prostate cancer is not readily predictable, and new diagnostics will be useful for treatment strategy determination. In this issue of the *JCI*, Haffner and colleagues use comprehensive tumor genome sequencing to investigate the origin of genetic mutations underlying a case of lethal prostate cancer. Surprisingly, the lethal clone in this individual arose from a tumor focus that is typically considered very low risk based on histology. Their report highlights the need to collect and curate “N of 1” cases to develop a database that can be used for clinical decision making.

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“N of 1” case reports in the era of whole-genome sequencing

A. Rose Brannon^{1,2} and Charles L. Sawyers^{2,3}

¹Department of Pathology, ²Human Oncology and Pathogenesis Program, and ³Howard Hughes Medical Institute, Memorial Sloan-Kettering Cancer Center, New York, New York, USA.

Prostate cancer has a range of clinical outcomes, from complete remission in response to treatment to death as a result of aggressive metastasis. Prognosis for individuals with prostate cancer is not readily predictable, and new diagnostics will be useful for treatment strategy determination. In this issue of the JCI, Haffner and colleagues use comprehensive tumor genome sequencing to investigate the origin of genetic mutations underlying a case of lethal prostate cancer. Surprisingly, the lethal clone in this individual arose from a tumor focus that is typically considered very low risk based on histology. Their report highlights the need to collect and curate “N of 1” cases to develop a database that can be used for clinical decision making.

The case of the lethal clone

Scientists are detectives at heart. When Haffner and colleagues learned about a case of lethal metastatic prostate cancer with evidence dating back 17 years, they had to take the case (1). Using an approach similar to one previously employed to follow pancreatic cancer progression (2), the authors began with comprehensive

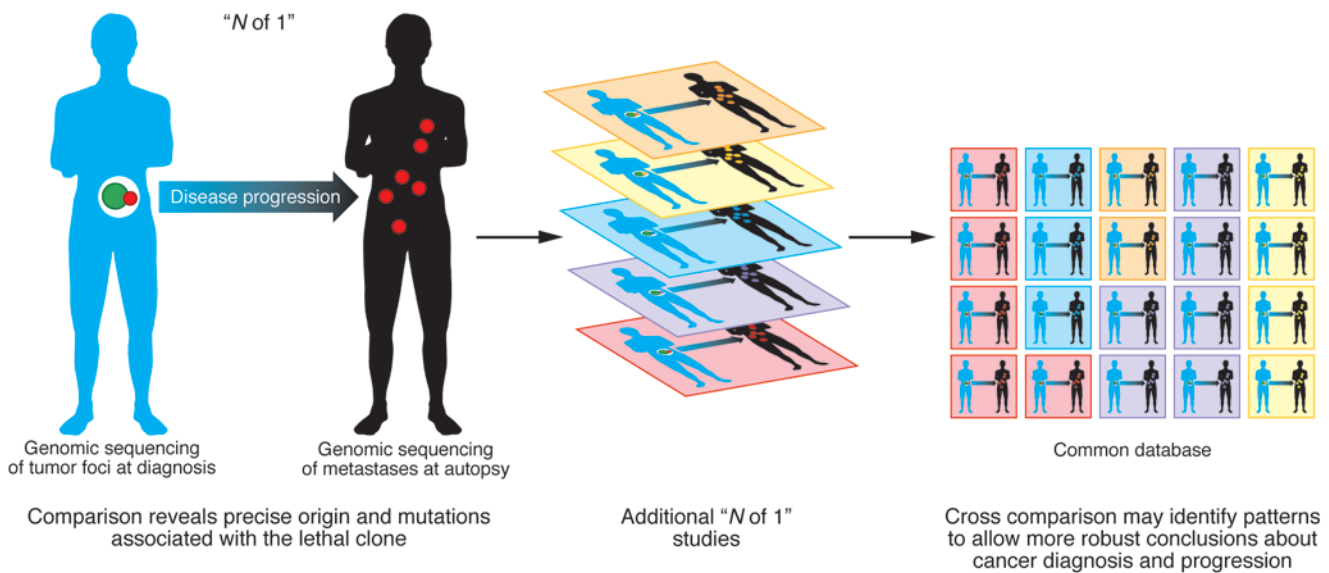
genome sequencing of metastatic tumor deposits recovered at autopsy. This analysis yielded evidence of mutations in several well-documented prostate cancer genes, such as tumor suppressor *PTEN*, tumor protein p53 (*TP53*), speckle-type POZ protein (*SPOP*), ATP-dependent helicase (*ATRX*), and androgen receptor (*AR*) (1), all of which are known to be recurrently altered in end-stage, castration-resistant prostate cancer. Because this patient’s primary tumor had been surgically excised (and saved) 17 years earlier, the authors had a unique opportunity to ask the “whodunit” question. Which, if any, of these mutations were present in the

primary tumor? How did this constellation of mutations evolve over time as the patient suffered multiple relapses (with accompanying tissue biopsies) during 17 years of treatment with various interventions? Answering these questions offered the potential for new insights into prostate cancer progression, drug resistance mechanisms, and perhaps opportunities to develop molecular diagnostics.

Histological examination of the primary tumor revealed multiple regions of high-grade (Gleason 4) tumor, a small focus of lower-grade (Gleason 3) disease, and a single lymph node metastasis (1). It is well established that patients with high-volume, high-grade primary disease have an increased risk of recurrence; therefore, this patient’s subsequent clinical course of metastatic prostate cancer is not surprising. It was surprising that the lethal clone, defined by the presence of the same *PTEN*, *TP53*, and *SPOP* mutations recovered at autopsy, originated from the small, low-grade Gleason 3 focus, and not from the much more substantial, high-volume Gleason 4 tumors, which did not harbor *PTEN*,

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**Figure 1**

The potential for "N of 1" studies to benefit clinical practice will require collection of these cases into databases. Genomics is a powerful tool for mapping tumor progression/evolution with great precision in individuals. Collecting these "N of 1" cases into a common database will help identify common patterns and enable more robust conclusions about cancer diagnosis and progression.

TP53, and *SPOP* mutations. Furthermore, the lymph node metastasis removed at the initial surgery was also negative for these mutations. Thus, this patient died from disease that, based on widely used (and validated) histologic criteria, would be considered low risk for metastasis.

Case closed?

We can certainly credit the authors for answering the "whodunit" question, but several clinical details of this case remain unexplained. For example, do the Gleason 3 (lethal) and Gleason 4 (nonlethal) foci represent independent primary tumors, or are they clonally related? This could have been addressed by comprehensive sequencing of multiple tumor foci, but the authors were unable to do so because of limitations in DNA quality (1). It is also unclear what molecular events led to the initial lymph node metastasis. Did this patient have a founder or "trunk" mutation present in all the primary tumor foci and the lymph node metastasis, with two distinct branches of tumor evolution, as recently described in kidney cancer (3)?

More importantly, are there broader lessons that we can take away from this case? For example, should we no longer trust the highly favorable prognosis associated with Gleason 3 cancer until we have evidence of a favorable molecular profile? If so, can we make a clinical decision based

on the molecular profile obtained from the biopsy of a single tumor focus, knowing that an entirely different profile might be seen from the biopsy of an adjacent lesion? Of course, it is impossible to make any such conclusions from a single case. Indeed, the academic community typically frowns upon clinical anecdotes, and for good reason: attempts to generalize lack scientific validity. Therefore, it is notoriously difficult to publish anecdotes, and most go unreported.

Despite the limitations of a single case study, enthusiasm for reporting single cases appears to be on the rise, as evidenced by other "N of 1" anecdotes in which comprehensive genomic sequencing has led to surprising insights. One recent example comes from tumor genome sequencing of an extraordinary responder to an experimental cancer drug. This patient was the only person enrolled in a clinical trial to test the mTOR kinase inhibitor everolimus in metastatic bladder cancer who had a complete remission (4). Remarkably, the patient's tumor had a mutation in *TSC1*, a tumor suppressor known to regulate mTOR kinase activity. Once this anecdotal evidence was obtained, the authors examined tumor tissue from other study patients. Notably, three patients whose tumors had *TSC1* mutations had partial responses, whereas all those whose tumors were WT for *TSC1* were unresponsive to

everolimus treatment. Despite the small number of patients, this report provided enough evidence for the commercial sponsor to initiate a new clinical trial of everolimus in *TSC1* mutant cancers, regardless of histology. Such biomarker-based clinical trials (called "basket studies") are becoming increasingly common in oncology and reflect growing confidence that molecular diagnostics can predict treatment response.

Future detective work

To harness the potential effect of these anecdotes on a larger scale, the National Cancer Institute recently announced the Exceptional Cases Initiative to identify and sequence the tumors from 100 extraordinary responders to any form of cancer therapy (5). If successful, this initiative could legitimize a new field of "N of 1" science. Should we also consider an initiative of "diagnosis-to-death" autopsy cases with longitudinal genomic analysis of tumor progression, as in the example reported here (1)? Such a project would be the 21st-century version of a much older, highly successful "initiative" led by William Osler and colleagues in the late 19th century, when autopsies were routinely performed and yielded new insights into disease pathophysiology. Importantly, these autopsies were not just a series of independent case studies. Only through the collection and



cross comparison of hundreds of cases did common patterns emerge, culminating in Osler's landmark text, *The Principles and Practice of Medicine*.

Haffner and colleagues should be commended for reporting such a fascinating anecdote (1), but the effect of their work will only be realized if we can collect and assemble this type of data across hundreds of cases (Figure 1). The growing penetration of genomic sequencing into clinical medicine has led to increased calls for the creation of various types of medical "data commons" that will allow comparisons between individual cases (6). Perhaps we are entering an era in which "N of 1" cases are here to stay.

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Address correspondence to: Charles L. Sawyers, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, New York 10021, USA. Phone: 646.888.2594; Fax: 646.888.2595; E-mail: sawyersc@mskcc.org.

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Antifibrotic vitamin D analogs

Joseph V. Bonventre

Renal Division, Department of Medicine, Brigham and Women's Hospital, Boston, Massachusetts, USA.

Chronic kidney disease is associated with progressive kidney fibrosis, which disrupts normal kidney function. There is a great need for treatments to reduce renal fibrosis. In this issue of the JCI, Ito and colleagues report the development of synthetic ligands of the vitamin D receptor that target the TGF-β-SMAD signaling pathway, which is known to regulate fibrosis-associated gene expression, without inducing VDR-associated genes. These ligands ameliorated renal fibrosis in two different mouse models. This study justifies further investigation of these and related compounds for treatment of humans with chronic kidney disease or other diseases characterized by fibrosis.

TGF-β signaling promotes chronic kidney disease

Chronic kidney disease (CKD) affects approximately 10% of the adult population in the developed world (1). A dominant feature of most forms of CKD is the development of kidney fibrosis, which results in progressive loss of kidney function, enhanced susceptibility to cardiovascular disease, and potentially end stage

renal disease. CKD progression occurs even if the original cause of the kidney disease is no longer operant. Despite the worldwide prevalence of CKD, few therapeutic strategies have any impact on the prevention or treatment of fibrotic kidney disease.

One factor with a prominent role in fibrosis development in the kidney and other organs is TGF-β, which signals through the TGF-β-SMAD signaling pathway (2). TGF-β binds to cell surface type I and II serine/threonine receptor kinases, resulting in phosphorylation of SMAD2 and SMAD3, which are then released into the cytosol and bind in a complex with SMAD4. After translocation to the nucleus, the SMAD2/3/4 complex localizes to SMAD-binding elements within the genome to modulate expression of profibrotic and other target genes (Figure 1 and ref. 3). During kidney injury, a major

source of TGF-β is proximal tubule epithelial cells, some of which are arrested in cell cycle phase G₂/M (4). The release of TGF-β and other factors by the damaged epithelial cells and infiltrating inflammatory cells act in a paracrine fashion to activate interstitial fibroblasts/pericytes. Once activated, these interstitial cells convert to proliferative myofibroblasts and maladaptively deposit extracellular matrix, which leads to interstitial fibrosis (2, 5). Since TGF-β plays a major role in this pathophysiological response, there have been attempts to therapeutically interrupt the TGF-β signaling pathway with either small molecules or antibodies. Some of these approaches are currently in phase I and II clinical trials (6); however, there is still no accepted TGF-β-targeted therapy for kidney fibrosis.

Vitamin D signaling in the kidney

Interaction of 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃] with the vitamin D receptor (VDR) modulates the transcription of more than 200 genes (7). The VDR forms a heterodimer with retinoid X receptors (RXRs) that together promote the recruitment of nuclear coactivators and the lysine acetyltransferase CBP/p300, which provides access for the basal transcriptional machinery through histone acetylation (Figure 1 and ref. 8). The VDR is expressed in more than 30 different tissues (9), including the kidney. VDR-

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