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Development of a translational medicine protocol for an NCTN genitourinary clinical trial: Critical steps, common pitfalls and a basic guide to translational clinical research

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Abstract

Translational medicine (TM) components of prospective clinical trials provide an invaluable opportunity to test hypotheses that contribute to our knowledge of human disease biology and/or the mechanism of action of a given therapeutic intervention. Our ability to sample tumors and their microenvironment, and the depth and breadth of biological information that can be extracted from them, has increased exponentially in recent years. This information is critical to guide the next steps clinical research if we are to accelerate the pace of progress in cancer treatment. Thus, TM studies should be considered key components of any clinical trial. However, TM studies are costly and biologic sampling can impose significant morbidity on our patients. Therefore, TM investigators should be engaged early in the design process (similar to a statistician) to ensure that the most imperative research questions are rigourosly defined, that the obtained specimens can be used to answer them and that the results will serve as the foundation for additional studies. In this review, we focus on TM studies in the context of the National Cancer Institute's National Clinical Trials Network trials and offer a description of the genesis of TM components, methods in sample acquisition and biomarker research, and a guide to funding mechanisms, in order to provide a blueprint for future TM research protocols. While TM studies can take many forms, the research discussed primarily focusses on basic and translational research involving molecular, cellular, and immunobiology. © 2018 Elsevier Inc. All rights reserved.

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1. Introduction

Translational medicine (TM) components of prospective clinical trials provide an invaluable opportunity to test hypotheses that contribute to our knowledge of human disease biology and/or the mechanism of action of a given therapeutic intervention. Our ability to sample tumors and their microenvironment, and the depth and breadth of biological information that can be extracted from them, has increased exponentially in recent years. This information is critical to guide the next steps clinical research if we are to accelerate the pace of progress in cancer treatment [1]. Thus, TM studies should be considered key components of any clinical trial. However, TM studies are costly and biologic sampling can impose significant morbidity on our patients. Therefore, TM investigators should be engaged early in the design process (similar to a statistician) to ensure that the most imperative research questions are rigourosly defined, that the obtained specimens can be used to answer them and that the results will serve as the foundation for additional studies. In this review, we focus on TM studies in the context of the National Cancer Institute's

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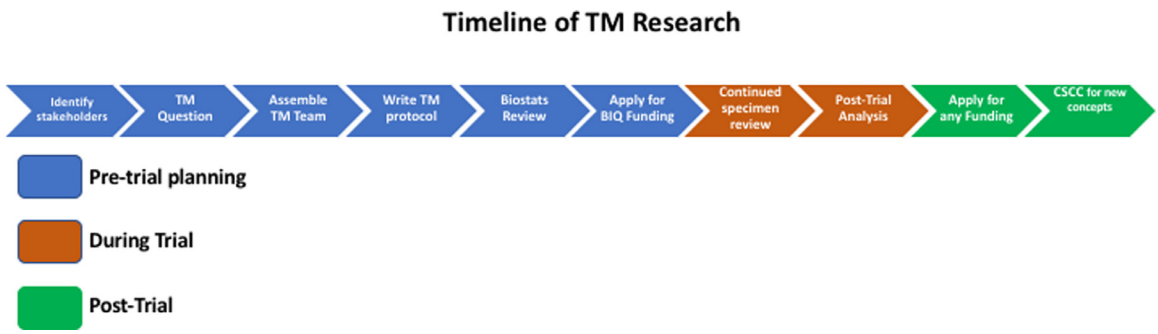

Fig. 1. Overview of the TM research timeline for an NCTN trial.

National Clinical Trials Network (NCTN) trials and offer a description of the genesis of TM components, methods in sample acquisition and biomarker research, and a guide to funding mechanisms, in order to provide a blueprint for future TM research protocols. While TM studies can take many forms, the research discussed primarily focusses on basic and translational research involving molecular, cellular and immunobiology.

2. The role of TM research in an NCTN trial

The TM component of an NCTN trial should develop in parallel with the conception of the clinical protocol (Fig. 1). Identifying the *specific questions* that can be asked in an NCTN trial and how that clinical outcome will be supported by biology, including biomarkers for response, are the foundation of TM research. At a practical level, the number of patients and corresponding biospecimens will affect the questions to be addressed.

3. Developing research questions

For NCTN trials, the TM research is centered around the major hypothesis of the clinical trial with both the clinical and TM questions directed towards hypothesis-driven research. Some general examples of TM questions include "if the clinical trial is successful, the mechanism of success is likely due to what cause and can we prove that at the cellular or molecular level" or "we think the patients in the trial who will respond the best to the treatment will express this biomarker." These research questions build the "Aims" of the TM protocol and should be distinct questions if possible, similar to the Aims of a grant application. TM Aims will often require multiple rounds of revision by the clinical, biostatistical, and TM teams to adjust the questions to fit the needs of the trial. While the platforms used to analyze the tissue samples will evolve over months and years, the main questions addressed by the Aims should remain important. Therefore, focusing on the research question has best opportunity for long-term success with the platform simply as a means to answer a question. Importantly, NCTN trials are not the right context for exploratory "hypothesis-generating" screens due to the critical value of human tissue and limited federal funding. Close collaboration with a cooperative biostatistical core is critical to a successful TM project to ensure the sample size of the trial will result in an answerable research question. For experiments involving NGS, the sample size of the trial is limited by the clinical outcome, but these may not be sufficient to answer a question with both discovery and validation cohorts. The level of significance will be dependent on effect size and biomarker accuracy.

4. The translational team

The collaboration of clinician/trialists, scientists, and biostatisticians involved in the development of a TM protocol is essential to construct a well-developed and relevant research protocol. This team is assembled at the time of protocol development and includes stakeholders with experience in the field of study. At least one member of the TM team should have experience in developing TM concepts, budgets, and applying for funding. The role of each team member is critical and often nonoverlapping. *The clinician* ensures the concept and question is both relevant and timely. *The trialist* ensures that specimens are obtainable, that is, patients will agree to give the tissues necessary to participate in research and the trial can be completed. *The scientist* will be essential to either answer the question or contract with other cores to move the research forward. *Patient representatives* play an important role in judging willingness of a patient to contribute biospecimens and participate in TM objectives. Finally, *the biostatistician* will determine if the question can be answered with a plausible sample size from the trial. If not feasible, the question or power for the entire trial needs to be adjusted or the question changed.

5. Biomarker research

The identification of biomarkers that are either predictive or prognostic is an important aspect of NCTN trials (Fig. 2) [2]. For inclusion in a clinical trial, a biomarker should have strong supporting prior data to justify its inclusion in a TM project. These biomarkers should have been evaluated in multiple retrospective settings, and the TM project is an opportunity for prospective validation.

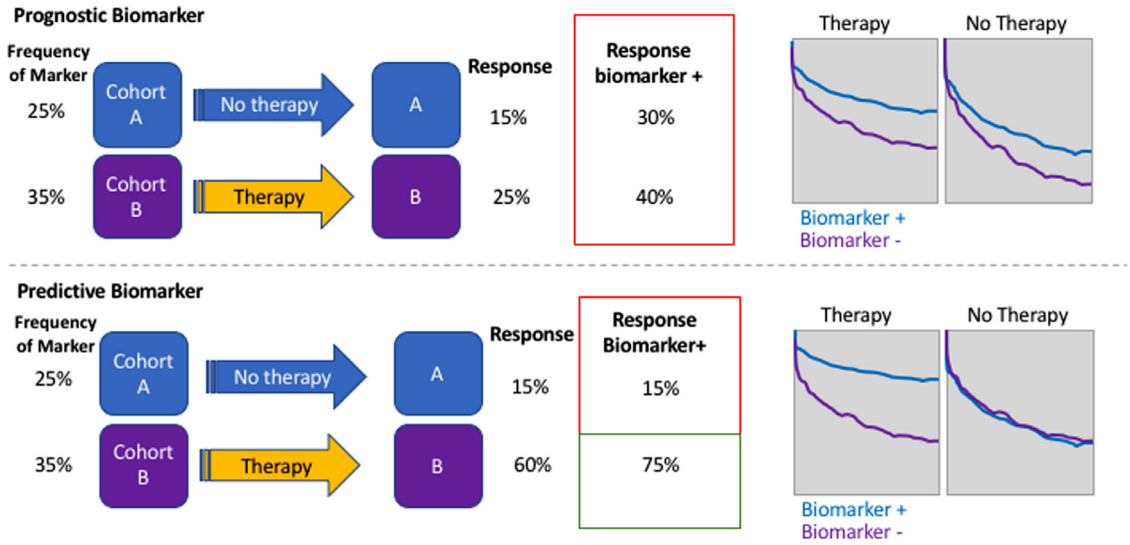

Fig. 2. The difference between prognostic and predictive biomarkers. Prognostic biomarkers (top) predict response or outcomes in both cohorts (A and B), independent of treatment. In this cartoon, the biomarker is present both groups and those with expressing a biomarker have a greater response, regardless of treatment (i.e. there does not appear to be an interaction of the biomarker with the treatment outcome). Predictive biomarkers (bottom) have an interaction, or a greater response in the experimental arm of therapy. In this example, patients in cohort B have a much greater response to treatment when they express the biomarker. Kaplan–Meier survival curves are shown with patients engaged in the study treatment compared to a control treatment. Patients that are biomarker positive have an improved outcome with a predictive biomarker. For prognostic biomarkers, survival is improved regardless of treatment.

Depending on the structure of the clinical trial, biomarkers should be classified as either *prognostic* or *predictive* (Fig. 2). Prognostic biomarkers provide information about the outcome of a patient (or cohort of patients) independent of the treatment arm. Prognostic biomarkers often relate to survival outcomes. For prostate cancer trials, PSA is strong prognostic marker because it predicts clinical outcomes for nearly all clinical treatments. A predictive biomarker refers to clinical response for only the treatment arm of a trial. For immunotherapy trials, tumor or immune expression of PD-L1 may be considered predictive of response to anti-PD-L1 therapies [3]. In general, predictive biomarkers are prioritized over prognostic markers because of their strong association with the trial and the experimental treatment. If validated, predictive biomarkers can directly translate into real world tests that guide patient decisions.

After a biomarker is determined to be predictive or prognostic, the next determination is how embedded the biomarker will be in the trial [4]. In the NCTN trials, the biomarker can be *integrated* or *integral*. Integrated biomarkers are evaluated during the normal course of the trial and do not directly impact patient assignment to treatment. In contrast, *integral* biomarkers define the clinical trial and determine which therapy is administered to participants. Treatment based on P53 expression to determine if a patient receives adjuvant chemotherapy is an example of an integral biomarker trial. For a biomarker to be *integral* in an NCTN trial, an assay has to meet clear criteria established by the NCI Clinical and Translational Research Operations Committee (CTROC) including performance in a CLIA approved lab, clear cut-points and reasonable turnaround time to inform therapies of a trial [5].

6. Budgets – funding the TM project

Funding of TM research is one of the most important challenges in clinical research and the responsibility of the TM team. While the clinical trial is often funded by a combination of standard-of-care (insurance) and NCI/CTEP, the TM component usually receives little to no funding through the NCI. Once approved by CTEP, most trials have a minimum budget that includes funding for obtaining slides for banking and resources for one tube of blood. The pharmaceutical industry is sometimes able to support TM research, especially for the development or validation of biomarkers that can support the application of their drugs. These relationships, once thought to negatively influence a trial, should be evaluated on an individual basis depending on the trial and company. With pharma involvement, the science should still be performed in an unbiased manner with unlimited access to the data for all involved from the TM team. The cooperative group contracting and legal services can ensure that the research will be performed and reported regardless of the trial outcome.

A more challenging situation occurs when no funding is provided. Therefore, these TM projects require competitive applications and grant support. For NCTN trials, an NCI BIQSFP (Biomarker, Imaging and Quality of Life Studies Funding Program) application is an outstanding mechanism that can fund integrated or integral biomarker research [6]. The goal of BIQ funding is to identify biomarkers that could shape clinical practice and these assays should be reliable and validated, with locked down cut points. BIQ funding applications are required within three months of review of the CTEP concept proposal. Important factors in

BIQ review are (1) validation of the biomarker, (2) applicability to the trial, and (3) platform rigor with possible commercialization. Given the demand for funding dollars, predictive biomarkers are prioritized over prognostic biomarkers. The BIQ application is reviewed by NCI Clinical and Translational Research Operations Committee (CTROC). In 2016, over $10 million was approved for funding. Most of the challenge of the BIQ is developing a fundable concept within the short-time from clinical trial submission. If the BIQ application is unfunded, many will not be funded on resubmission. BIQ applications that are descriptive or propose discovery research have a very low likelihood for success.

Foundation support (from organizations like the HOPE Foundation) offers an unprecedented opportunity for funding support of TM research [7]. The HOPE foundation supports research through the Southwest Oncology Group (SWOG) and provides multiple funding mechanisms through the year including SEED and Impact grants that are similar to R03 and R21 mechanisms from the NIH. These grants are competitive, and even an Impact award is not sufficient to complete a TM project but would be sufficient to submit a high-quality grant with preliminary data.

If industry and foundation support are not successful, extramural grant funding is the next step in funding TM research. The modular (base) budget for a five years R01 begins at $1.25 million dollars, and the use of specimens from an NCTN trial often improves the impact score of a grant. Specific Requests for Applications (RFAs) are flexible and the reader should consult the current list of RFAs posted by the NCI. The Department of Defense, CDMRP has a funding mechanism similar to a small P01 grant called a "Team Translational Science Grant [8]." This mechanism involves two and three PIs whose work is synergistic and based largely on a clinical trial. This is an outstanding opportunity and we hope that the CDMRP will continue to offer this mechanism of TM grant support.

7. Cores of specialization

The NCI has recently supported the development of Cancer Immune Monitoring and Analysis Centers (CIMACs) that will conduct focused immunotherapy support for NCTN trials [9]. The overarching goals of these centers is to identify molecular biomarkers that define immune response and support specialized centers of excellence for NCTN trials. CIMACs are supported by a U24 mechanism with the goal of (1) predicting the likelihood of benefit or toxicity from immune therapies, (2) helping to develop rational combination therapies to overcome intrinsic or acquired resistance,(3) better understanding resistance mechanisms, and (4) providing monitoring strategies and evaluating target inhibition via pharmacodynamic biomarkers. Four cores were approved in 2017 (MDACC, Dana Farber Cancer Institute, Stanford University and Mount Sinai). Each core has nonoverlapping strengths and goals to provide support for clinical trials. Through a separate mechanism, the Dana Farber was awarded a cancer immunology data commons grant to provide repository and centralized support and integration of the CIMAC groups.

8. Managing trial patient bioresources

The identification of what biologic resources are necessary to answer TM questions is a fundamental question in TM protocols. Specimens are often collected at the time of registration, but they can also be collected during the course of a trial. Coordination of shipping, including batching of samples may require orchestration with the biobank and individual sites. The TM coordinator will need to develop collection kits and/or specific shipping directions that can be implemented across sites. In GU trials, these specimen sources include the tumor, germline DNA, urine, and blood with timing of specimen acquisition potentially occurring before and after treatment. As the era of liquid biopsy and next-generation sequencing evolves, the current leading-edge platforms may not be relevant when the trial is complete. Nevertheless, the principle of cataloging and storaging tissue and blood is enduring and agnostic of the platform. With cost as the major limitation, we recommend banking as many biospecimens as may be justified.

During protocol development, an important consideration is whether the specimen is required or optional. Trial participants are encouraged to allow use of unstained FFPE slides from their tumor. We recommend a slide depth of 5 to 10 μm depending on the application. Alternatives to slides include paraffin ribbons or block punches. The TM protocol also includes banking instructions for the cooperative group biobank. Once slides are received at the biobank, they are barcoded and stored under conditions that depend on the potential long-term goals. Urine and blood can be processed by the donor site given that freezing and thawing may disrupt cells and proteins. Urine is usually spun to separate the cells, and for trials involving immune responses in the bladder, saving these cells may be important. For cell-free DNA in urine, we separate the sediment-free soluble fraction from the urine and it is frozen for later analysis. The blood is processed on site with heparin tubes and frozen for DNA or separated in plasma fractions for cell-free DNA. Unlike tissue, which is usually either fixed or frozen at the time of collection, blood presents additional logistical challenges for sites. For plasma-based assays, the blood requires centrifugation prior to storage. For cell-based assays (i.e. for analyses of circulating tumor cells for somatic mutation calling or white blood cells for germline), there may be additional steps like Ficoll spins or processing in real time through CTC enrichment or identification and recovery platforms. Depending on the downstream assays intended (plasma vs. cells, DNA vs. RNA), different collection tubes with or without preservatives may be needed. These may need to be supplied to participating sites in advance in prepacked collection/mailing kits. Given the

importance of these bioresources, communication with individual sites and the cooperative group biobank must continue at regular intervals throughout the trial. Some patients may refuse to have specimens banked because they are concerned about the extra time, cost, and anonymity. Thus, we recommend direct contact of the TM coordinator with the site study lead and nursing coordinator to ensure as many bioresources as possible are obtained.

Once the trial concludes, use of specimens not described in the TM protocol approved by the NCI requires approval of parent cooperative group and the NCTN's Clinical Correlative Science Committee (CSCC). This requires formal approval by the NCTN Statistical and Bank review for concept feasibility. Concepts approved then undergo NCTN Group Scientific Review and independent review by CSCC. If approved, the proposal finally requires IRB, MTA, and conflict of interest review and approval prior to distribution of specimens. In addition to the multiple levels of approval, funding for the research will also need to be obtained.

9. Conclusion

The TM component of an NCTN trial complements the clinical trial and can provide important information that directly affects patient care and future trials. Barriers to TM research are the amount of resources and funding that are necessary to complete TM research projects. A carefully developed TM strategy can change clinical care and provide and unprecedented avenue of future of discovery.

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