“Triple Negative Breast Cancer”: Translation Research and the (Re)Assembling of Diseases in Post-Genomic Medicine

Peter Keating\textsuperscript{a}, Alberto Cambrosio\textsuperscript{b}, and Nicole Nelson\textsuperscript{c}

\textsuperscript{a}Department of History, University of Quebec at Montreal
\textsuperscript{b}Department of Social Studies of Medicine, McGill University
\textsuperscript{c}Department of the History of Science, University of Wisconsin, Madison

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Abstract:
The paper examines the debate about the nature and status of “Triple-negative breast cancer”, a controversial biomedical entity whose existence illustrates a number of features of post-genomic translational research. The emergence of TNBC is intimately linked to the rise of molecular oncology, and, more generally, to the changing configuration of the life sciences at the turn of the new century. An unprecedented degree of integration of biological and clinical practices has led to the proliferation of bio-clinical entities emerging from translational research. These translations take place between platforms rather than between clinical and laboratory settings. The complexity and heterogeneity of TNBC, its epistemic and technical, biological and clinical dualities, result from its multiple instantiations via different platforms, and from the uneven distribution of biological materials, techniques, and objects across clinical research settings. The fact that TNBC comes in multiple forms, some of which seem to be incompatible or, at least, only partially overlapping, appears to be less a threat to the whole endeavor, than an aspect of an ongoing translational research project. Discussions of translational research that rest on a distinction between basic research and its applications fail to capture the dynamics of this new domain of activity, insofar as application is built-in from the very beginning in the bio-clinical entities that emerge from the translational research domain.

Keywords:
Translational research; oncology; breast cancer; molecular diagnosis; targeted therapies; genomics

Application is not extrinsic to modern knowledge, it is not just added to some epistemic core; it exerts its action at the very level of concept formation itself; the technical belongs to the essence of the modern sciences themselves.

Rheinberger (2005, p. 324)

Introduction

During a June 2012 meeting devoted to National Institutes of Health (NIH) funding, the US Senate Appropriations Committee expressed its concern “about the toll of triple negative breast cancer” [henceforth TNBC] and urged the National Cancer Institute (NCI) to collaborate with other organizations “to help improve treatment and survival rates” (Bin Han Ong, 2012). A decade before, the Committee would most likely have expressed concern over the high rate of breast cancer in general, rather than a specific subcategory of the disease. Recourse to TNBC itself would have been impossible since the disease did not then exist. Its rise to Senate-level prominence was thus relatively swift. A search in PubMed shows that the first article using the term TNBC in its title or abstract did not appear until 2007 when it also entered the public domain, showcased as a national problem in O, The Oprah Magazine (Fischer, 2007; see also Okura, 2010). The year before its PubMed consecration, friends of a young woman diagnosed
With TNBC at age 35 had established *The Triple Negative Breast Cancer Foundation*.\(^1\)

While mass media and policy forums reacted promptly to the emergence of this new disease, TNBC’s status within biomedicine remained controversial, as evidenced by article titles such as “Triple-negative breast cancer: disease entity or title of convenience?” (Carey et al., 2010), or “Triple-negative breast cancer: making the most of a misnomer” (McCarthy et al., 2012). At a 2013 breast cancer conference, a leading clinical researcher stated categorically that TNBC was not a *bona fide* disease and that speakers should avoid the term ... a statement that did not prevent other speakers from using it, with apologies, throughout the conference (fieldnotes, IMPAKT 2013 conference, 2-4 May 2013). At the 2015 edition of that same conference, as part of a session specifically devoted to TNBC, the pathology presenter stated authoritatively that TNBC was “merely an operational term covering a collection of heterogeneous diseases” (fieldnotes, IMPAKT 2015 conference, 7-9 May 2015). Despite questions concerning the definition, status, and in some cases the very existence of TNBC, by 2014 the *Clinicaltrials.gov* website (the U.S. “registry and results database of publicly and privately supported clinical studies of human participants conducted around the world”) listed about 240 studies devoted to the disease. Indeed, its widespread clinical use had already prompted a team of European clinicians to publish an article entitled “Triple negative breast cancer: proposals for a *pragmatic definition* and implications for patient management and trial design” (Eiermann et al., 2012; our emphasis). All of this suggests that even in an evidence-based, research-intensive domain such as oncology it remains possible to study and treat diseases that large sectors of the medical community consider misnamed, purely conventional, or even non-existent.

TNBC can be deployed as an object of practical clinical concern *and* as a target of biological investigation (an “epistemic thing”). Consider, for example, the 2013 meeting of the American Society of Clinical Oncology. As evidenced in the meeting abstracts, clinical researchers framed TNBC in multiple clinical and research contexts, and used it to investigate its clinical and pathological behavior, to compare it with other kinds of breast cancer, to calculate the rate of hereditary mutations it harbors, to study the response of its subgroups to traditional and novel (“targeted”) therapies, to combine it with other subtypes of breast cancer in order to establish prognostic and predictive subsets, as a starting point for the discovery of yet other breast cancer subgroups, and to investigate its molecular pathways and markers both because they might predict response to therapy *and* in order to unravel TNBC’s peculiar biology. In other words, TNBC was deemed an entity worthy of investigation on its own, and as an operational category at the service of a higher calling, the improvement of cancer therapy. Both are ways of saying that TNBC is (clinically) useful, and both are interconnected, as the improvement of cancer therapy these days depends upon knowledge of the mechanisms that inhabit and animate the entities treated.

Research along these lines proceeds unabated at the time of this writing. While some teams continue the search for prognostic and predictive TNBC gene signatures (Pinto et al., 2016; Liu et al., 2016), the Intensive Trial of OMIcs in cancer (ITOMIC), a distributed clinical research

\(^1\) [http://www.tnbcfoundation.org/ourstory.htm](http://www.tnbcfoundation.org/ourstory.htm)
network centered on the molecular features of cancer, selected metastatic TNBC for its first clinical trial to exemplify the network’s “intensive longitudinal monitoring” approach (Blau et al., 2016). As for the Translational Research Network in Oncology (TRIO) — a “worldwide network of 2,000 Investigators located in 500 research centers residing in 45 countries spanning 5 continents” — it is looking at repurposing drugs to treat TNBC, i.e. drugs that failed previous tests due to possible problems with the high-throughput methods used in their evaluation (Slamon, quoted in Nailor & Lewis, 2016). In January 2016, the aforementioned Triple Negative Breast Cancer Foundation joined forces with Carol’s Crusade for a Cure Foundation (another private charity devoted to “raising awareness and funding to support organizations at the forefront of [TNBC] research”), and most importantly, with the American Association for Cancer Research, to announce a new grant opportunity for “basic, translational, or clinical” research on metastatic TNBC, explaining that “this type of cancer is a particularly aggressive form of breast cancer for which there are no targeted therapies”.

These activities, which link practical clinical concerns with biological investigations, take place within a number of programs and networks that, as we just saw, explicitly refer to translational research [henceforth TR]. Moreover, several key protagonists of the TNBC domain (e.g., Nielsen, 2010; more on this below) conceive of themselves as translational researchers. A case study of TNBC will thus provide relevant evidence for the investigation of the concrete research practices (as opposed to policy statements) that characterize TR as defined by the actors themselves. Since its introduction at the US National Cancer Institute in the early 1990s in connection with the characterization of breast cancer susceptibility genes (BRCA), the term has become ubiquitous in biomedical debates. It is generally taken to refer to major investments in biomedical infrastructures, training, and research to help cross a perceived gap (“the valley of death”) between laboratory research and clinical applications (Butler, 2008), but its exact meaning and the practices it entails or ought to entail are open to debate. Several policy reports (for a review, focusing on the UK, see Morgan et al., 2011) and articles, some of which in aptly named journals (e.g., Drolet & Lorenzi, 2011; Mankoff et al., 2014) have advocated a number of different means to steer and promote TR, often represented as a flow (unidirectional or bidirectional) between laboratory and clinical settings. Others have advocated initiatives aimed at establishing appropriate infrastructures and reward systems for what they consider as a new research domain (e.g., Hood 2008). On the more analytical side, researchers have provided scientometric evidence of the emergence of a TR domain as characterized at an aggregate level by distinctive citation and semantic networks (Cambrosio et al., 2006; Jones et al., 2011); they have investigated the dynamics of the organizations involved in TR, for instance the existence of a “hidden research system” in universities and academic hospitals (Lander & Atkinson-Grosjean, 2011); and of scientific-regulatory hybrids (Kohli-Laven et al., 2011); and they have examined

2 http://www.cirg.org/html/investigator.html
4 Present-day translational research, and its stated goal of realigning biology and the clinic, can be located in a specific historical conjuncture. While the aftermath of World War II — a period retrospectively referred to as the “golden years” of clinical research (Swazey & Fox, 2004) — saw the emergence of the physician-researcher, the years since have been marked by the rise of molecular biology, and the physicians who had initially launched the clinical research revolution slowly became outnumbered by Ph.D.’s with no clinical experience (Ahrens, 1992).
how researchers and clinicians situate themselves vis-à-vis the institutionalization of this new sphere of activity (Morgan et al., 2011; Vignola-Gagné, 2014; Lander, 2016).

Critics have argued that TR, as described in the aforementioned contributions, is “merely” a policy object (some would even say: a buzzword), or at best a peculiar set of institutional arrangements, with no distinctive epistemological quality. In other words, old wine in new bottles, as links between bench and bedside have been around for long time, in particular at institutions such as the NIH where research laboratories rub shoulders with a major research hospital. This argument, however, depends on maintaining a dichotomy between organizational and cognitive/epistemic components of biomedicine which is dubious at best (see Cambrosio et al., 2014 for a detailed discussion of the relationship between the organizational and epistemic features of oncology research). Historical studies of the emergence of biomedicine and the biotech industry (Gaudillière, 2002; Löwy, 1996; Rasmussen, 2014) have likewise pointed to the existence, well before the 1990s, of many instances of investigations combining practical clinical concerns with the production of biological knowledge, which substantially resemble activities that are today identified as TR. While these critiques about the degree of continuity or discontinuity of contemporary practices are well taken, an unprecedented degree of integration of biological and clinical practices has more recently led to the proliferation of bio-clinical entities of the kind that will be discussed in this paper. Most importantly, our focus on the objects and entities emerging from TR has led us to examine translations taking place between platforms (Keating & Cambrosio, 2003) — such as the work done to bridge traditional pathological techniques with more recent high-throughput technologies — rather than translations between clinical and laboratory settings. While studies of human practitioners have often postulated the existence of an essential tension between researchers and clinicians (e.g., Hedgecoe, 2003; Timmermans & Buchbinder, 2011; Quirke and Gaudillière, 2008, p. 451), we treat platforms as bio-clinical assemblages which belong from the beginning to both treatment and research. Translations taking place between platforms, then, are not processes or sites where the laboratory and the clinic are merely interfaced, but where existing bio-clinical entities are reworked and remixed, both conceptually and materially, generating the proliferation of definitions and uses we see in the case of TNBC.

Our focus on platforms and the objects of TR also allows us to account for another aspect of the TNBC trajectory. The opening paragraphs of this article highlighted the controversial status of TNBC as a bio-clinical disease entity. As the rest of this paper will show, TNBC is not only a disputed entity, but also a rapidly changing one. The existence of competing definitions of an entity such as TNBC is less an expression of different schools of thought, or thought styles, or paradigms — to borrow terminology from the last century — than the expression of a need to define for specific conventional purposes exactly which instantiation of this nosological entity — which platform for (re)producing it — is being used at any given time.

**TNBC and the rise of molecular oncology**

TNBC arose at the intersection of two lines of work. The first, pathology, has in recent decades
supplemented its traditional focus on the visual inspection of tissues with the adoption of immunohistochemistry (henceforth: IHC) and, subsequently, molecular-biological techniques such as FISH (fluorescence in situ hybridization), while maintaining close connections to clinical activities and routines. The second, genomics, is more commonly associated with TR, and thrives on the deployment of the latest “high-throughput” technologies, in particular gene expression profiling with microarrays, and more recently DNA and RNA sequencing. These two lines of work have led to a number of (sometimes controversial) attempts to align their results, both conceptually and technically.

*Figure 1* reprinted from an article entitled “Lost in Translation?” (Harbeck & Rody, 2012, p. 688), depicts the progressive dismantling of a common pathology — breast cancer — into an increasing number of rare diseases. This process relied on two mutually reinforcing events: (a) the deployment of a number of platforms that redefined tumors in terms of biomarkers, beginning first with single biomarkers (the ER and PR hormone receptors, and HER2), moving on to gene expression profiles defining a set of “intrinsic subtypes” (e.g., Luminal A, Basal-like), and to the analysis of DNA mutations and molecular pathways; and (b) the development of new chemotherapeutic agents that selectively target tumors harboring specific receptors and biomarkers (hence their “targeted therapies” designation). We say “mutually reinforcing” because the characterization of tumors in terms of biomarkers has led to the design of new drugs targeting specific patient subpopulations, and, conversely, the development of drugs that selectively work on subpopulations of clinical trial patients has given rise to the recognition of biologically distinct categories of tumors. There was, in other words, a direct connection between biological and clinical work, and this is why we speak of bio-clinical objects and entities.

**FIGURE 1 ABOUT HERE**

As noted by Foulkes et al. (2010), the definition of TNBC as a disease first appeared in the medical literature in 2005, in a contribution by Brenton et al. (2005) that freighted the term from the very outset as both a biological and a clinical matter of concern. The authors of that paper employed the term in three different ways. They first used it as a clinical qualifier with therapeutic consequences for another, recent subtype of breast cancer (basal-like breast cancer) introduced in the year 2000 thanks to the use of a new genomic technology for analyzing gene expression (microarrays): “given its triple-negative receptor status ..., basal-like breast cancer is not amenable to conventional targeted therapies for breast cancer ... leaving only chemotherapy in the therapeutic armamentarium”. Later on, they deployed the term as a qualifier for patients: “triple-negative patients”, and finally, in the conclusion, they evoked the existence of (patients with) “a triple-negative cancer”, thus defining, albeit almost as an afterthought, a new type of cancer.

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5 The definition of a biomarker is itself controversial. As noted by Marchiò et al. (2011, p. 41), “biomarkers are the defining facet of translational cancer research; however, there is a great deal of confusion about the actual definition of what a biomarker is and what its characteristics are”. 

Use of the three receptors to describe a class of patients preceded the act of naming the disease. In 1998, for example, a large retrospective study identified two cohorts of patients on the basis of negative or positive receptor status, showing that the one with a positive receptor had a better response to a drug called doxorubicin (Paik et al., 1998). The term “triple-negative”, however, did not itself appear in the paper. Prior to 2005, then, there were triple-negative patients (i.e., triple-negative was a classifier) but no triple-negative breast cancer (i.e., triple-negative was not yet a distinct disease). Ultimately, the appearance of TNBC as a specific category in 2005 required the emergence, five years earlier, of a new experimental classification scheme that simultaneously negated the autonomy of TNBC by conflating it with one of its components, basal-like cancer. To see how this was possible, we need a closer look at the trajectories (first separate, then interwoven) of the three receptors and of gene-expression subtypes.

**What’s in a name? A tale of three receptors**

The designation “triple-negative” refers to the absence of three cellular receptors — ER, PR, and HER2 — in breast cancer tissue samples. The first two receptors, ER and PR, are hormone (Estrogen and Progesterone) receptors. Awareness of their clinical importance as indicators of patient response to hormone therapy is generally traced back to the 1960s. But since the 1990s, ER and PR have also acted as markers of a biological subcategory of the disease. The third receptor, HER2, is a cell surface receptor discovered in the mid-1980s. It was used throughout the 1990s as a breast cancer prognostic indicator, before becoming a predictor of response to the new anti-cancer drug trastuzumab (one of the poster children of “targeted therapies”) that gained FDA approval in 1998. How the receptors became markers requires some explaining that takes into account the techniques used to make them visible, and the evidential contexts to which they were successively related.\(^6\)

While ER emerged as a clinical object more than 50 years ago, many aspects of its current use are relatively novel. It was only in the 1990s, for example, that all-comers breast cancer clinical trials were replaced by trials that accrued patients with a specific combination of markers, in recognition of the fact that, for instance, ER-positive and ER-negative breast cancers should be considered different diseases. Concurrently, pathologists using IHC took control of ER-testing, which was previously under the purview of clinical biochemists using radioactive assays. Thus, whereas ER began its existence as a clinical object in the 1960s when initial reports linked estrogen receptors to the fact that some patients responded to hormone therapy while others did not (Folca et al. 1961), it underwent a profound transformation in the 1990s when it was translated into a full-fledged bio-clinical entity.

The transformation began in the 1970s when initial indications that hormone receptors might predict treatment response were consolidated in an NCI symposium on the *Prediction of*

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\(^6\) The techniques for determining ER and PR are basically the same and have evolved similarly, but while they intersect, the trajectories of the two receptors differ somewhat. For brevity’s sake, we will here focus on ER.
Response in Cancer Therapy (Jensen et al., 1971). This insight gained clinical status in 1974 when the Breast Cancer Task Force of the NCI collated accumulating data on endocrine treatment response and its relation to ER status, and concluded that ER status “should permit the practicing oncologist to select or reject endocrine therapy with considerable confidence” (McGuire, 1975). In 1979 a NIH consensus conference recommended that ER analyses be performed on all primary breast cancer patients. While retrospective accounts present these events as a linear sequence of increasingly robust guidelines, major uncertainties continued to surround the status of ER in the 1970s. Endocrine therapy remained unspecific and included not only chemical but also surgical techniques, i.e. oophorectomy. Moreover, there were persistent problems with the biochemical measurement techniques: competing methods and different cut-offs led to contradictory results (Hawkins et al., 1980).

Uncertainty continued into the 1980s. A 1986 review, for instance, reverted to the conditional form when stating that the “application of an estrogen receptor scheme (positive vs. negative) for defining breast cancer may allow delineation of cases that may appear quite similar but that represent two different types of disease” (Stanford et al., 1986). The review further noted that it had “not been established whether estrogen receptor-negative tumors represent an advanced stage of the disease or arise de novo”. The 1980s, however, also saw the staging of a landmark clinical trial (NSABP’s Protocol B-14) that firmly rooted ER in the therapeutic realm. Begun in 1981 and completed in 1988, the trial focused on the use of tamoxifen in ER+ patients, providing evidence of the benefits of this drug in this specific patient population (Fisher et al., 1989). In this respect, ER acted as an indicator of response to a specific therapy (tamoxifen) and not as a marker of a biologically distinct disease. In other words, the trial turned ER into a bona fide clinical object or, to use a now fashionable category, a clinically useful one. The transformation of ER into a bio-clinical object had to wait until the subsequent decade and the intervention of pathologists.

While initial studies of ER used biochemical assays to measure levels of the receptor and relate them to response to hormone therapy (Walker, 1999), the introduction of IHC allowed pathologists to visualize ER on fixed tissues, opening up new investigational avenues for the study of the etiology and bio-pathology of breast cancer. It was not an easy transition. As late as 1996, the clinical practice guidelines issued by the American Society of Clinical Oncology recognized the entrenched status of biochemical testing by recommending it as the preferred method for routine clinical use (ASCO, 1996) even though such tests presented their own challenges. Specifically, biochemical measurement of ER demanded a “technically challenging and expensive” technology that required radioactive reagents and fresh-frozen tissue (Harvey et al., 1999). Fresh-frozen tissue was relatively rare as pathologists (who as tissue curators and sole experts in tissue analysis act as gatekeepers for accessing tissue samples) work overwhelmingly with paraffin-embedded tissues. Moreover, biochemical testing involved “grinding-up” the tissue prior to evaluation, thus preventing visual inspection of the distribution of ER receptors in biopsy samples, a key component of the pathologists’ skill set. Finally, the determination of the presence or absence of ER receptors was a quantitative undertaking, further excluding pathologists unfamiliar with quantitative biochemical approaches (Interview, January 2012). By the early 1990s, however, the availability of IHC established a bridge between ER and
pathologists, who were now able to use antibodies to stain tissue samples, observe the results under a microscope, and, most importantly, use reagents on paraffin blocks, the standard means of preserving biopsy specimens.

Pathologists, however, did not simply “take over” from clinical biochemists: they had to adjust their practices. The introduction of IHC was a mini-revolution for a specialty that until the end of the 1970s had been based on the observation of the morphology of cancer tissues under a microscope (Soilleux & Gatter, 2006; Crompton, 2011). Furthermore, as noted by a leading breast cancer pathologist (Interview, January 2012), the fact that by the late 1990s breast cancer clinical trials began being routinely tailored towards biomarker-defined subpopulations of patients, led to the realization that the accuracy and reproducibility of those measurements in different pathology laboratories was far from perfect, and that this state of affairs “was deleterious for the patients ... and ... it was jeopardizing the results of the trial”. This led to further transformations of pathology’s practices via the staging of interlaboratory standardization and quality control initiatives, and the widespread adoption of auditing practices such as central pathology review to verify the results submitted by local pathology laboratories (Cambrosio et al., 2015). In sum, it is fair to say that ER was still relatively new in the year 2000 (Harvey et al., 1999).

By the beginning of the 1990s, rather than simply handing off a case to the treating physician, pathologists had become increasingly involved in therapeutic decisions. This transformation was captured by a series of interviews published in the professional journal of U.S. pathologists (Chapman, 1991), where one pathologist ascribed this new form of cooperation to the recent availability of new molecular methods:

> Being able to tell, through molecular efforts, what’s there, how much, where it is, and what it’s doing is going to revolutionize pathology’s role, especially in oncology. Not only will pathologists be called to surgery, but I believe we will be the physicians’ instrument in individualizing treatment protocol for each tumor patient. [p. 29; our emphasis]

Yet another recounted how breast cancer pathology lay at the vanguard of the “revolution”. Whereas pathologists had previously been content to describe, they now attempted to prescribe:

> Now we not only say it is cancer, but if it is, for example, breast cancer, we will also describe what hormone receptors it has, how much DNA is in the average nucleus, what oncogenes are activated, what stage of the cell cycle the cancer cells are likely to be in, whether it is growing rapidly or slowly and what may be the most effective chemical or hormonal therapy. [p. 25; our emphasis]

This transformed the relations between clinicians and pathologists, as shown by the following interview excerpts with two leading breast cancer pathologists:

> For 50 years we were uninterested in the developments taking place in oncology ... [my
teachers and mentors’] interest was pathology for pathologists: let’s get really accurate in what we do ... and that led us to produce reports that at some point in the 90s were two pages long, with information that was of little use for clinicians. And now, since the beginning of the 2000s, we’ve been asked to do the opposite; [clinicians tell us:] “make the reports really concise, synoptic reports only with [only the] information we need”. (Interview, May 2013)

Whenever I have residents discussing with me, I will always tell them: “Listen now, you prepare your report. Before signing it out, you have to relax and read your report, and then try to figure out the prescription. If you are able to do that, the report is correct. If you are not able to do that, there is something wrong with your report”. (Interview, January 2012)

In contrast to ER, HER2 emerged as a full-fledged bio-clinical object in the post-biotech era (Rasmussen 2014; Hughes 2011). Iterations of HER2 were developed in different experimental systems in the first half of the 1980s, and by 1985 a consensus was reached that those different entities were one and the same (Coussens et al., 1985). It was also concluded that HER2 was amplified in breast cancer cell lines. Clinical work began at about the same time when a Genentech researcher who had isolated the gene for HER2 struck an agreement with an UCLA oncologist, the former supplying biological probes and the latter access to his collection of tumor specimens. HER2 stood out from the rest in breast and ovarian cancers, but given that the UCLA tumor collection lacked patient histories for the breast cancer samples, the team turned to a San Antonio researcher who had a substantial collection of frozen tumors replete with case histories (Bazell, 1998, pp. 36-39). Notice the strategic role played by access to clinical material. Increasing awareness of such a role would subsequently lead to calls for the establishment of biobanks as components of an expanding bio-clinical infrastructure (Gottweis & Petersen, 2008).

While early research pointed to a significant correlation between HER2 amplification and disease progression, and thus to its prognostic value (Slamon et al., 1987; Cline et al., 1987), investigators in both Europe and the United States initially failed to confirm those findings (van de Vijver et al., 1988; Ali et al., 1988). Some suggested that one of the reasons for this might be that, rather than a simple prognostic indicator, HER2 amplification signified the presence of a distinct type of breast cancer (van de Vijver et al., 1988). In any event, a 1995 review of prognostic research concluded that a “true idea” of the role of HER2 had yet to emerge (Ravdin & Chamness, 1995). As most of the studies had used IHC techniques, and in the absence of settled conventions for staining and scoring, the results offered no clear direction: in fact, HER2 assessment turned out to be even more complex than ER measurement.

HER2’s status changed significantly in 1998 when in addition to a marker, it became a target. Genentech (working again with UCLA) gained FDA approval for the anti-HER2 targeted-therapy drug trastuzumab (commercially known as Herceptin; see Bazell, 1998). HER2 over-expression, in addition to being a prognostic indicator, thus became an indication for the use of trastuzumab. Within the context created by this dual role (Dixon et al., 2012), the debate
continued over the exact means of HER2 testing (Allison, 2010), with pathologists and oncologists producing competing society guidelines (Schmidt, 2011).

The development of trastuzumab profoundly modified the therapeutic landscape: breast cancer patients with positive hormone receptors would be offered endocrine therapy, while hormone receptor negative patients testing positive for HER2 could now be treated with trastuzumab. Only “triple-negative patients” were thus left with few therapeutic options. This situation became further problematized as an “unmet medical need” (Hudis & Gianni, 2011), a phrase that far from being purely descriptive was an official designation calling for political and regulatory intervention.7 Does this then account for the emergence of TNBC as a distinct clinical entity? In other words, should we argue, as a leading clinician did (interview, June 2011), that TNBC “is definitely not a biological entity ... it’s a practical clinical entity based on practicality, which means that we need to find treatments for it”? Not quite, as the concept of TNBC as a disease did not emerge until 2005, well after the advent of trastuzumab, for reasons that cannot be reduced to pragmatic considerations.

From immunohistochemistry to gene profiling: microarrays enter the stage

To account for the appearance of TNBC we need to make allowance for the initiation of a second line of work at the beginning of the new century. Molecular biologists, quickly joined by a novel brand of molecular pathologists, began to churn out breast cancer classifications based on newly available genomic tools, such as microarrays. In so doing, they created the problem of aligning old and new understandings of a disease and, in the case of breast cancer, of a disease that was in the process of fragmenting into a number of distinct diseases.

As we saw above, TNBC was first used as a qualifier and then as a proxy for basal-like breast cancer (henceforth BLBC). The latter owes its existence to the deployment of the aforementioned microarrays that analyze gene expression. First made available during the second half of the 1990s (Cointet et al., 2012), a microarray experiment involves the simultaneous analysis of many hundreds or thousands of genes, as opposed to a single gene as was then common in molecular genetics. While more recently dislodged by next-generation sequencing technologies (Nelson et al., 2013), microarrays were initially hailed as the tool of choice for the genomic analysis of biological and biomedical specimens (Mogoutov et al., 2008; Rogers & Cambrosio, 2007). Central to the development of this new tool was work performed in the middle of the 1990s in Patrick Brown’s lab at Stanford. Brown quickly initiated collaboration with David Botstein’s lab, also at Stanford. Botstein’s lab had been involved in the development of trastuzumab with Genentech, and he was thus quite aware of the genetic heterogeneity of breast cancer.

7 The official definition by the FDA is worth quoting: “‘An unmet medical need is a condition whose treatment or diagnosis is not addressed adequately by available therapy. An unmet medical need includes an immediate need for a defined population (i.e., to treat a serious condition with no or limited treatment) or a longer-term need for society (e.g., to address the development of resistance to antibacterial drugs).’” (FDA, 2014, p. 4)
Botstein and Brown thus set out to use microarrays to explore the molecular diversity of breast cancer (the results of which they termed “molecular portraits”), beginning with samples sourced from local surgeons. Charles Perou, a postdoc, joined them in this endeavor and became the first author of a widely cited 2000 Nature paper (over 5,500 citations by the end of 2014) that classified breast cancer according to gene expression patterns into four biological (not clinical) “intrinsic subtypes” (Perou et al., 2000). The term “intrinsic” was a direct reference to the fact that the subtypes were held to correspond to the fundamental biology of breast cancer, rather than simply matching gene expression with clinical outcomes, as was the case with commercial gene expression tests being developed at around the same time (Kohli-Laven et al., 2011). The complementary issues of the clinical validation of the subtypes and their clinical utility then moved to center stage.

The first microarray studies of breast cancer conducted by the group compared tumor tissue with human cell lines and rendered two breast cancer subgroups: one with a high level of proliferation genes and one with a high level of transcription genes. The work was co-authored by a Stanford pathologist, Matt van de Rijn, experienced in the use of IHC techniques. As a result, the Stanford team used commercially available antibodies that translated results from one technology into the other as a way of validating the new tool. The microarrays used in the initial experiments, however, did not include genes for ER or HER2 despite their role in breast cancer biology, for the goal of the initial project was to show only the “feasibility and usefulness” of the new approach (Perou et al., 1999).

Although tumor samples for the initial experiments from the Stanford area were in limited supply, thanks to a visiting Norwegian oncologist the team gained access to an annotated collection of fresh-frozen samples from a number of studies carried out in Bergen. Having proven to their satisfaction that the new approach was sound, the team modified the protocol by increasing the number of genes on the microarrays from 5,000 to 8,000 and by including the well-known tumor drivers HER2 and ER. The results were four clusters: a Luminal/ER gene cluster, a cluster overexpressing HER2, and two “basal-like” clusters that became one shortly thereafter, in a 2001 paper based on a new series of 78 tumors (Sørlie et al., 2001; approx. 4,400 citations by the end of 2014). The now unified basal-like cluster correlated with clinical data showing that the subtype had a poor prognosis.

The Stanford team now had to validate the new classification, and this required access to relatively large collections of clinical material. There were two problems in this regard. Given the shortcomings of the US health-care system, it was difficult to access and assemble patient materials linked with outcome data, especially from patients treated in a consistent way. Moreover, archived patient material usually presented itself in the form of formalin-fixed samples, amenable to IHC analysis, but not to microarray testing that required fresh-frozen tissue. Collaborations with clinical researchers from Germany, Norway, and, in particular, Canada solved the first problem. The second necessitated the translation of the microarray intrinsic subtypes into their IHC equivalents. In other words, the development of an IHC version of the subtypes was not an afterthought that sought to apply knowledge of a biological entity — the subtypes — to the clinic, or to develop a commercial product that could find an easy way
into the clinic: it was necessary for the validation, and thus the constitution of the subtypes as *bona fide* divisions of breast cancer. In this respect, a decisive encounter between Perou and a British-Columbia pathologist, Torsten Nielsen, took place at the 2000 meeting of the American Association for Cancer Research where Perou showcased his initial results in a poster presentation.

Both as an MD-Ph.D. surgical pathologist who, “as a pathologist ... had access to tissue and ... could see where the diagnostic problems were”, and as a self-defined translational researcher with an interest in both sarcoma and breast cancer (Nielsen, 2010), Nielsen was immediately drawn to Perou’s work. He visited Stanford shortly after the meeting and underwent training with the team’s pathologist, van de Rijn, who also happened to be an early adopter of another technique, tissue microarrays, popularized at the end of the 1990s (Kononen et al., 1998). Not to be confused with DNA microarrays, tissue microarrays consist of glass slides spotted with a number of small histologic sections from conventional paraffin-embedded tissue blocks that display tissue from multiple patients or blocks on the same slide. Nielsen went back to Vancouver with “marching orders” to find tissue linked to outcome, a task facilitated by the fact that the BC Cancer Agency maintained a provincial database of thousands of cancer specimens. He soon discovered a collection of specimens that allowed him to produce a first series of tissues microarrays with approximately 900 patient samples (Nielsen interview, April 2014). Two aspects of this initial work bear emphasis. First, intrinsic subtypes qualified as bio-clinical entities from the very outset insofar as they mobilized novel biological laboratory platforms that were triangulated with pathology’s IHC techniques, and, second, their use entailed clinical material in association with clinical annotations.

The intrinsic subtypes went through a number of versions over the years as additional tumor samples were analyzed: the luminal cluster was divided into Luminal A and Luminal B, and two new (albeit minor) clusters were added, named “Claudin-low” and “Molecular apocrine”. Perou moved to North Carolina (UNC) where he enjoyed joint membership in the departments of genetics and pathology, as well as membership in the local cancer center, thus (not unlike Torsten Nielsen) adding an institutional dimension to his TR practices. Having a “vested interest in trying to develop and provide the next generation of molecular pathology assays for oncology”, he also realized that although oncologists order the tests, pathologists run and interpret them (Perou, interview, July 2010), which meant that the platforms on which the tests would run needed some adjustment. The NCI apparently shared these concerns, since, in 2004, it initiated the Strategic Partnering to Evaluate Cancer Signatures (SPECS) program to support multi-institutional, multidisciplinary research teams involving biotechnology companies, community hospitals, national laboratories, and academic institutions, to develop “robust, reproducible assays” for molecular signatures derived from the molecular analysis of tumors. A team consisting of Perou (UNC), Nielsen (UBC) — who had in the meantime discovered another treasure trove of about 19,000 breast cancer samples (of which about 3,500 could be cross-referenced with outcome data) —, as well as colleagues from Washington University, the University of Utah, and an oncology cooperative group (CALGB) who specialized in biostatistics, bioinformatics, and another key molecular biology technique, the polymerase chain reaction (PCR), successfully applied to the SPECS program.
PCR was chosen as the preferred option, because it could be adapted to the pathologists’ paraffin-embedded samples (like IHC and unlike microarrays), and be used for the simultaneous testing of multiple genes (like microarrays but unlike IHC). Moreover, seen from the pathology lab, PCR enjoyed a more robust status than the experimental microarray technology. In the course of development, the team narrowed down the list of genes necessary to distinguish between intrinsic subtypes to 50, produced a PCR-based version of the test called PAM50 (Parker et al., 2009), and established a company, Bioclassifier, to handle the intellectual property. Finding a commercial partner was part of the mandate of the SPECS program, but this turned out to be more complex than anticipated. Lack of space prevents us from discussing those intricacies here. In the end, a commercial version of the test was eventually marketed by the company Nanostring under the name of Prosigna, using a different technology (the company’s nCounter system) which can be used in every pathologist’s lab. After extensive validation, the test was cleared by the FDA in 2013 (Nielsen et al., 2014).

At this point it might be asked, as indeed it was, whether the molecular portraits of breast cancer delivered anything new. The answer depends on the terms of comparison. For instance, if compared to traditional morphologic pathology (Ellis et al., 2003), the two classifications are hardly identical, as shown by Figure 2. Moreover, the success of the intrinsic subtypes papers, as measured in terms of citations, is proof enough of novelty, and, one may add, of the “revolutionary” character of the application of genomic technologies to oncology.

If, however, one compared the intrinsic subtypes to the results produced by pathologists who by then routinely used IHC to analyze receptors, the intrinsic subtypes appeared at once compelling and mundane. A 2009 review noted: “in hindsight, this molecular taxonomy provided little additional insights to the standard subdivision of breast cancer for therapeutic purposes” (Weigelt & Reis-Filho, 2009, p. 722). Notice the “therapeutic purposes” clause: the issue is not presence or absence of novelty in the abstract, but in relation to the therapeutically useful. A 2012 European task force that investigated the medical usefulness of the intrinsic subtypes (and in particular the PAM50 assay) concluded that it remained inadequate, as it did “not provide sufficiently robust information to modify systemic treatment decisions”, although it “should ... be incorporated into clinical trial design” for research purposes (Guiu et al., 2012). Criticisms like these relied on empirical studies (for a review see Weigelt et al., 2012) that, turning the tables showed that even though IHC’s lack of standardization may have enticed researchers to embrace gene expression profiling for its promise of increased objectivity, that approach suffered similar problems. In particular, with the notable exception of basal like cancers, assignment of tumors to intrinsic subtypes appeared to be method-dependent, and the subtypes themselves to be “unstable”.

Between the enthusiasts and the skeptics lay those who recognized the stimulating effect the intrinsic subtypes had had on the field of breast cancer research despite their limited penetration into routine diagnostics. A long review of microarray breast cancer research in the
International Journal of Surgical Pathology concluded that while it was indeed “unlikely that microarrays themselves will ever become one of the main tools for making decisions about treatment for breast cancer patients”, they had “led to a paradigm shift in the way breast cancer is perceived and how breast cancer research is carried out” (Correa Geyer & Reis-Filho, 2009, p. 302). In fact, the success enjoyed by the intrinsic subtype terminology left one leading breast cancer pathologist complaining that pathologists had come to use the terminology even in the absence of any gene expression analysis: in particular, fellow pathologists mixed terms and tools by speaking of “basal-like” (a gene expression profiling definition) instead of “triple negative” (the IHC definition) to report on results obtained with IHC (Fieldnotes, Conference on “Prognosis and Prediction in Breast Cancer”, Monaco, October 2008). According to the same speaker, this was no minor terminological issue as TNBC (as defined by IHC) and basal-like tumors (as defined by genomics) differed significantly. As we will see in the next section, the relation between TNBC and BLBC became a key focus of research. But first, a few more comments on the attempt to find IHC surrogates of the intrinsic subtypes.

Why would anyone want to use older, established technologies such as IHC, when novel, technologies are at hand? This mixture of the mundane and the disruptive relates to the need to create continuity between novel entities and existing clinical categories and practices, which is both a practical concern for clinical activities, and, in the case of molecular tests, a possible determinant of commercial success or failure (Kohli-Laven et al., 2011). As previously noted, initial attempts by the Stanford team to find IHC correlates for the intrinsic subtypes were prompted by the need to validate those subtypes using patient samples, rarely available in the fresh-frozen format required by microarrays. This was particularly true for the basal-like subtype, which, unlike the other subtypes, stood out as a “previously unrecognized” subclass (Alizadeh et al., 2001, p. 51). In collaboration with Nielsen, Perou developed an IHC equivalent of basal-like (Nielsen et al., 2004) as part of the more general endeavor to validate, characterize and qualify the new entities, in particular their prognostic value, by triangulating them with existing biomedical entities and tools.

The team deployed a panel of four antibodies “routinely used in the clinical setting” with tissue microarrays that, as we saw, came with clinical annotations: two antibodies for “negative” purposes, i.e. to detect the absence of ER and HER2, and two to detect the presence of two other biomarkers: cytokeratin 5/6 and EGFR. The latter were less straightforward than one might initially surmise. Cytokeratin was considered specific though not very sensitive insofar as it picked out only half of the basal-like tumors. EGFR was neither specific nor sensitive, but a marker of bad prognosis. There was also a treatment-related rationale for its inclusion: although it was “not a basal-like cancer specific marker”, it was a target for a first generation of targeted agents (i.e. gefitinib and erlotinib, mostly applied to lung cancer), and so the marker “could be used “to define a subset of breast cancer patients who might benefit from treatment with one of those agents” (Nielsen et al., 2004, p. 5373).

Perou’s move to North Carolina opened up the possibility of answering questions that could not be answered with the UBC material. UNC had a unique collection of breast cancer specimens that contained samples of pre-menopausal African American women, a class of cancer patients
with a notoriously poor prognosis for reasons that were then unclear. Teaming up with Lisa Carey, a UNC medical oncologist, and using a modified version of the IHC test that added PR antibodies to the mix, their study found that African American women were twice as likely to fall into the basal-like category than the general population (Carey et al., 2006). Perou’s IHC panel included tests for ER, HER2 and now PR, and these were all expected to be negative in the BLBC cases examined. In this roundabout way, the triple-negative definition was now embedded in the basal subtype through the redefinition of the IHC version.

The IHC instantiation of basal-like breast cancer attracted mixed valuations from pathologists in what can viewed as yet another staging of the mundane and the disruptive. Those who saw the work as something new, a “potentially important tumor subset”, also viewed the results as “inherently descriptive”, despite the inclusion of prognostic markers, and as little evidence for any conclusion on the existence of a new cancer type (Gusterson et al., 2005). Others viewed the work as a contribution to a line of inquiry already underway within pathology, as work on those markers and their expression in normal and tumor cells could arguably be traced back to the early 1980s. Studies by a number of research pathology collectives had either disaggregated the basal type into several subtypes (which meant that “basal-like” was an “oversimplification” and that “basal cancers are heterogeneous”; Jacquemier et al., 2005, p. 267), found other morphological markers to identify it (Matos et al., 2005), or identified the basal type as an already known morphological form (Reis-Filho et al., 2006). Last but not least, measurement of some of the IHC markers was particularly problematic. As Perou himself admitted, “the keratin 5/6 is just a very hard marker to score pathologically. And so, I just didn’t feel comfortable trying to use that as a clinical decision maker, and so I left it at that” (interview with Charles Perou, July 2010).

And yet, attempts to reduce the intrinsic subtypes to IHC measurements have continued unabated. A case in point is the St. Gallen International Breast Cancer Conference, established in 1978. One of the key biennial events in breast cancer oncology, St. Gallen is known for its respected (albeit often controversial) cancer treatment consensus recommendations. The 12th edition of the conference took place in 2011 under the motto “strategies for subtypes” (Gnant et al., 2011) and endorsed the use of the intrinsic subtypes of breast cancer to simplify the definition of therapy indications. Recognizing, however, that it was often difficult to test for subtypes clinically using genomic technologies, they recommended a “simplified classification”, in fact a translation from microarray technology to IHC, as a “useful shorthand”. While it acknowledged that “subtypes defined by clinicopathological [i.e. IHC] criteria were similar to but not identical to intrinsic subtypes”, the St. Gallen recommendation maintained that the IHC surrogates nonetheless represented “a convenient approximation”. Basal-like was thus translated into ER, PR, and HER2-negative. Although the St. Gallen “shorthand” seemed to equate “triple negative” with “basal-like”, the text of the recommendations added that the overlap between the two entities was only 80%, and that “triple negative” was in fact a heterogeneous category, including mostly cancers with a bad prognosis, but also low-risk histological subtypes that could be diagnosed through traditional visual inspection of tissue morphology.
Another major event in the trajectory of the basal-like subtype had occurred eight years before, when studies on tumors associated with the breast cancer hereditary susceptibility gene, BRCA1, showed that they fell within the basal-like cluster (Sørlie et al., 2003; Foulkes et al., 2003). Basal-like thus came to describe a subgroup of cancer that had clinical importance because of its dire outcome either on its own terms or because of its association with BRCA1. Initially confined to the hereditary cancer, the domain of BRCA was considerably widened in 2004 when research expanded into sporadic (somatic) cancer through the notion of BRCAness (Turner et al., 2004), which denotes a BRCA-like behavior caused by alternative mechanisms in the absence of the corresponding mutation (Bourret et al., 2014). Connections between BRCA, TNBC, and Basal-like breast cancer multiplied thereafter. The 2005 paper that introduced TNBC (Brenton et al., 2005) defined, *inter alia*, a basal phenotype as “one of the hallmark features of “BRCA-ness”. One year later Haffty et al. (2006) showed that “patients with BRCA1 mutations develop predominantly triple negative tumors”. Subsequently, a team of French pathologists even suggested that basal-like breast cancers be renamed “triple-zero/BRCAl like” (Vincent-Salomon et al., 2010). *Figure 3* provides a synthetic view of the entanglements between BLBC, TNBC, and BRCA circa 2010. Each of these bioclinical entities, as we will see in the next section, simultaneously stabilized and disrupted the others.

FIGURE 3 ABOUT HERE

TNBC made and remade

The relation between TNBC and BLBC — their status as mutual proxies and their imperfect overlap — quickly became an ongoing concern. In 2007, a team from the Dutch National Cancer Institute selected triple-negative samples on the basis of IHC, profiled the samples with microarrays, and claimed to have found that triple negative tumors were quite simply “synonymous with basal-like tumors” (Kreike et al., 2007, p. 1). Indeed, in the years that followed its baptism in 2005, early inquiries into TNBC took for granted that almost all of the cases were, in reality, basal-like. A 2008 review of the field maintained that while TNBC was a “slightly less accurate but reasonable proxy for basal-like breast cancers”, at least “80% to 90% are basal-like” (Peppercorn et al., 2008). Expanding on this theme in an interview in 2008, Carey explained:

The term “triple-negative” is used to mean a particular molecularly identified subtype, the basal-like subtype. And while it is true that almost all triple-negative breast cancers are basal-like, it’s also clear that not all of them are. Some basal-like breast cancers actually have hormone receptors or HER2/neu positivity. So, these aren’t perfect proxies for one another. And, right now, the methodologies that we have are good at identifying hormone receptors and HER2/neu expression; they’re just not great at identifying basal-like breast cancers. (Carey cited in Berman, 2008; see also Irvin & Carey, 2008)

Others, taking microarray studies as the gold standard, described TNBC not only as a proxy but also as a “poor man’s” definition of the real thing (Linn & van’t Veer, 2009). And yet, the
relatively easy access to TNBC via IHC opened the doors to retrospective studies and clinical trials. The first attempts were somewhat hesitant, as when TNBC appeared in scare quotes in the title of an abstract presented at the 2006 San Antonio Breast Symposium (Garber et al., 2006), or as in 2007 when it was described as the “so-called triple negative phenotype” (Bauer et al., 2007). But subsequent studies became increasingly assertive. Figure 4 shows the growth in the number of breast cancer publications using the term “triple negative” in their titles or abstracts, confirming Prat et al.’s (2014) regretful acknowledgment that “over the years, BLBC has become more commonly known as TNBC”.

**FIGURE 4 ABOUT HERE**

Examples of early clinical studies include a Toronto investigation of a cohort of TNBC patients extracted from a collection of slides from the Women’s Hospital between 1987 and 1997, and stained for the three markers in the period 2000-2004. Based on the assumption that “the ‘basal-like’ category of tumors is composed almost entirely of ‘triple-negative’ breast cancers” (Dent et al., 2007), this study was followed by a similar one in North Carolina using samples collected at the local cancer center defining once again “the basal-like subtype as ER-, PR- and HER2-” (Carey et al., 2007). Yet another retrospective study performed at M.D. Anderson analyzed response to therapy and long-term survival of TNBC patients (identified via IHC), once again equating TNBC with BLBC (Liedtke et al., 2008).

In spite (or because) of the undeniable clinical uptake of the new disease category, some pathologists and oncologists began voicing a more critical position, questioning the equation between TNBC and basal-like, or even more radically, questioning the existence of TNBC as a *bona fide* nosological entity. In their opinion, the overlap between basal-like and TNBC was not only incomplete, but also sufficiently limited to warrant a clear distinction between these two entities. In a letter castigating the authors of the aforementioned Dutch reverse-engineering paper who had concluded that basal-like and triple-negative tumors were synonymous, a group of prominent breast cancer oncologists and pathologists stated in no uncertain terms that equating TNBC with basal-like was simply “misleading”, noting that the overlap between the two entities involved a game of shifting percentages. ER expression by IHC, they noted, was to be found in 5% to 45% of basal-like tumors as defined by microarray, and HER2 overexpression in 5% to 15% of basal tumors (Rakha et al., 2007). The task was thus to “unravel” or “dissect” the complexity of both TNBC and basal-like tumors (Metzger-Filho et al., 2012). A variety of actors, including molecular biologists, pathologists, and clinicians, took part in these debates, with fault lines emerging not only between but also within categories, and with arguments ranging from the biological to the therapeutic consequences of a given stance.

A detailed critical review published in 2008 examined the heterogeneity of BLBC and concluded that in spite of “distinctive morphologic, genetic, immunophenotypic [IHC], and clinical features”, no “accepted consensus on routine clinical identification and definition” of BLBC was available, nor was there “a way of systematically classifying this complex group of tumors” (Rakha et al., 2008, p. 2572). This was not simply a problem of diagnosis: it also had clear therapeutic implications, as inconsistent diagnoses might “hamper consistent identification and
development of treatment strategies for these tumors”. Indeed, a table in the paper listed 11 clinical trials allegedly involving BLBC but where, in 10 cases, the definition of basal-like corresponded in fact to triple-negative, a definition that “may arguably lead to inaccurate conclusions as a result of the noise in subtype definition introduced by relying on a triple-negative criterion”. The concluding recommendation was clear: “studies analyzing triple-negative tumors should be labeled as such, whereas the definition used for basal-like phenotype should be clearly stated in those dealing with basal-like breast cancers” (Rakha et al., 2008, p. 2572).

Informal discussions on the sidelines of the annual United States and Canadian Academy of Pathology meeting, a conference that attracts mainly research pathologists, led to the drafting of a position paper on BLBC and TNBC (interview with Jorge Reis-Filho, May 2013). Published in Modern Pathology and co-signed by 19 pathologists from the U.S., Europe and Asia, the paper functioned as a sort of advisory to pathologists and oncologists, stating that because “it does not lead to any direct clinical action”, and given the variations in its definition, the “use of the term ‘basal-like breast cancer’ in diagnostic surgical pathology reports does not appear to be justified”. Adopting a pragmatic clinical attitude, the authors added that “perhaps more important than identifying the basal-like subgroup within triple-negative breast cancers [was] the identification of subgroups of triple negative disease that are sensitive to specific systemic therapy regimens” (Badve et al., 2011). This last injunction underlines a well-known fact: the important features of any entity are those that are held to be interesting. In the case of TR, mechanisms of therapeutic sensitivity and resistance are intrinsically more interesting than the multiplication of subgroups.

Indeed, as should by now be clear, the TNBC/BLBC nexus is more than a nosological curiosity, partly due to its clinical implications, in particular the fact that TNBC designates a category of patients for whom there is no effective treatment. In the era of molecular therapies, however, no sharp divide can be drawn between molecularly derived nosology and therapy. TNBC has become the starting point for an increasing number of bio-clinical investigations, and although initially defined as equivalent to BLBC, it has clearly acquired an independent existence. In fact, recent work on the molecular characterization of TNBC has inverted its relationship with BLBC. A team from Vanderbilt-Ingram Cancer Center isolated six different molecular subtypes of TNBC including two that are basal-like (Lehmann et al., 2011). Rather than a subset of basal-like, in other words, TNBC contained subsets of basal-like tumors. In a partial rebuttal of the Vanderbilt analysis, Perou and Carey, in collaboration with translational genomics colleagues from Barcelona (Prat et al. 2013), argued that the Vanderbilt classification rested on three subtypes that were, in fact, “very concordant” with PAM50 subtypes. As illustrated in Figure 5, they thus proposed a subdivision of TNBC into basal-like and non-basal-like tumors, insisting on the clinical implications of this dichotomy. Readers will notice that Figure 5 includes tentative therapeutic indications, and that the authors also argued that clinical trials for TNBC should be sufficiently powered to detect differences in response between the two main subgroups.

FIGURE 5 ABOUT HERE
The interweaving of the biological and the therapeutic does, however, raise difficult issues. While heterogeneity makes TNBC an interesting research subject, the multiplication of subtypes could leave practicing oncologists in a quandary, as the exact consequences of the expanding nosology remain unclear. When asked by The ASCO Post in the course of a 2012 interview how many types of cancer there might be, George Sledge, Past President of ASCO and Co-director of the Breast Cancer Program at Indiana University replied:

It depends on whom you talk to. For instance, breast cancer has what are widely recognized as four or five intrinsic subtypes. But Dr. Jennifer Pietenpol and her colleagues at Vanderbilt University have said that one of those subtypes—triple-negative breast cancer—can be further divided into another six subtypes. That’s 15% of breast cancer cases with six different subtypes at a molecular level.

Sledge then went on to ponder the clinical consequences of this proliferation of subtypes:

At some level, the question becomes, how do you define a subtype? In theory, every patient could represent his or her own subtype, because everyone has different mutations. ... But is it important from a treatment standpoint? A lot of the supersubtypes may not be particularly important from a treatment standpoint, in part, because we haven’t yet identified the mutations [that drive the cancer development]. (Bath, 2012)

To sum up, and as shown by Figure 4, the number of papers with the term “triple-negative” in their titles or abstracts has continued to grow, overtaking basal-like publications. Perhaps even more telling, the intersection between these two sets is about 11%. TNBC attracted the attention first and provided the context for the discussion. And even though its heterogeneity denies it the status of a unique biological category, there is nothing to prevent an in-depth discussion of “the biology of TNBC”. In 2012 TNBC received a (yet again pragmatic) sanction from the U.S. FDA Draft Guidance on the use of pathologic complete response as a primary endpoint for clinical trials seeking accelerated approval in cases of high-risk, early stage breast cancer (FDA, 2012). The only type of cancer explicitly mentioned in the Guidance was TNBC and, accordingly, industry observers saw the Guidance as a means to “jump-start” development of therapies for TNBC. In an interview with BioCentury, representatives of the FDA admitted that: “this is the population the agency had in mind when it drafted the guidance” (McCallister, 2012). One can speculate whether this has connections, direct or indirect, with the Senate Committee hearings and the Triple Negative Breast Cancer Foundation we mentioned at the very outset of this paper. While the molecular dissection of breast cancer has not led to an equivalent fragmentation of patient advocacy groups and dedicated charities — the broader entity, breast cancer, still serves as the rallying point — an “intermediate” entity like TNBC can act as a focus for specific initiatives.

As a bio-clinical object TNBC has retained an epistemic relation with the arguably more fundamental, albeit less practical, category of BLBC (which means that it also shares the unknowns of that category), and with the entire realm of biological research that describes the processes that give rise to that phenotype. This is not an atypical situation. In fact, almost any
clinical category is surrounded, so to speak, by a host of animal models, cell lines, and research hypotheses that act as proxies or avatars for the disease within clinical cancer research. Continued research can either stabilize or upset the category along any number of lines. The dual nature of the bio-clinical object thus prompted the question, raised at a 2009 conference on advances and controversies in breast cancer, of “whether TNBC is a distinct pathological subtype of breast cancer or a pragmatic category for determining eligibility for clinical trials and guiding individual patient treatment” (Carey et al., 2010, p. 683). The open-ended answer provided by practitioners was that it was “neither a single disease entity nor a title of convenience”, but from a sociological point of view one could as well have argued that the answer was: both. Indeed, its overlap with BLBC reinforced its biological/epistemic nature, while its widespread clinical acceptance justified its routine use. These problems, as we saw, have not stopped research on TNBC, and thus TNBC’s existence as a bio-clinical object of inquiry. It would be trite to say that TNBC is a victim of its own success if only because the point of research into TNBC is not to save the phenomenon, but to unravel the processes that underlie it.

Conclusion

The complexity and heterogeneity of TNBC, its epistemic and technical, biological and clinical dualities, result from its multiple instantiations via different platforms, and from the uneven distribution of biological materials, techniques, and objects within the TR space. They are also the result of a series of historical contingencies that produced the new disease entity first by way of a negative definition — the absence of three markers routinely inspected by pathologists — then through the entrenchment of the disease in medical practice. Numerous attempts to reconcile evidence that the new entity lacked coherence with its widespread use have so far failed. Clinical researchers, however, seem to consider the fact that TNBC comes in multiple forms, some of which seem to be incompatible or, at least, only partially overlapping, less as a threat to the whole endeavor, than as an aspect of an ongoing TR project. In other words, they appear to have implicitly adopted Kripke’s (1980) view of reference according to which scientific terms are historical, not logical constructs. According to this picture, referents begin their semantic trajectory within a community through an original description that, for a time, fixes the object. Subsequent research might significantly modify the description to the extent that the items originally involved in the description could be shown to be falsely associated with the term. In other words, “later empirical investigations may establish that some of the properties did not belong to the original sample” and that, conversely, “an item may possess all the characteristics originally used and fail to belong to the kind” (p. 137). Thus, “reference is determined by a causal (historical) chain, not by use of any of the items” (p. 139). As shown in this paper, understanding innovation in the life sciences clearly requires taking into account both the shifting content of bio-clinical practices and entities, and the temporal order in which they intersect and assemble (Bourret et al., 2014). Given the constant evolution of knowledge in post-genomic medicine (and the many dead ends it entails), the interest in any bio-clinical entity rests partly on the significant unknowns that it contains. As noted in our introductory remarks, the existence of competing definitions of TNBC can be accounted for by the need to define in pragmatic terms which platform for (re)producing TNBC is being used at any given time, while
generating further questions as to the clinical and biological make-up of that entity.

Our analysis of the TNBC trajectory allows us to direct attention to what appears to be a blind spot of current discussions of TR, which rest, implicitly or explicitly, on a distinction between “basic” research and its applications (hence, the need for “translation”). The epigraph at the beginning of this paper, excerpted from a discussion of Bachelard’s notion of \textit{phénomenotechnique}, suggests otherwise. As Rheinberger notes (2005, p. 324), Bachelard did not endorse “the idea of a science in search of application” but sought instead to describe:

a science that is taken and accepted as science because it moves in and has always existed in the realm of the applicable, because its very epistemological constitution has a technical dimension, because application is built into the very meaning of concepts and into the rules of concept formation, because the technical is built into the experimental phenomena, and because, just the other way around and in a symmetrical fashion, the noumena are built into the instruments and take on an instrumental form that further serves to develop the whole phenomenotechnical machinery.

Biomedical research material in oncology, such as TNBC samples, is by definition human, their tissues and/or parts, and (animal) models thereof. Results obtained using these materials are directly relevant (and therefore “applicable”) to the understanding of human health and disease. In this sense, results made visible in one tissue or disease are then, in principle, immediately available to all those with access to patient samples. The “in principle” clause renders these observations somewhat problematic, because as we have shown the availability of tissue collections or lack thereof played a major role in selecting key participants in the TNBC saga and distributing roles among them. And yet, the availability of the material needed to produce phenomena across the entire space of TR clearly undermines the notion of separate zones of experiment and application. Research is always already applied insofar as it bears upon objects of clinical and biotechnological concern. Conversely, clinical research simultaneously treats epistemic objects insofar as the targets of therapy are bio-pathological constructs.

There is a second, less obvious sense in which biomedical objects of research have built-in applicability, for the TNBC case study clearly shows that the phenomena to be studied cannot be usefully separated from the techniques that bring them into view and allow them to be manipulated. A number of different platforms are available at any given time, whose results do not always or necessarily align (Keating & Cambrosio, 2003). In the case of TNBC, tissue staining and light-microscopy, immunochemistry, gene expression profiling with microarrays, DNA and RNA sequencing, and, more recently, epigenetics and proteomics, all serve to identify various aspects of TNBC, and all these platforms are correlated with clinical findings. Practitioners have no trouble using different technical descriptions of what they consider the same, albeit controversial object. In this paper we have focused on how, in practice, researchers and practitioners manage different uses of TNBC in the rich and diverse sphere of translational activities, and thus generate a set of coherent practices, in spite of the lack of coherence of TNBC as the entity on which those practices converge.
While the emergence and development of TR can be tracked by focusing on the rise of a new kind of clinician-researcher or by following the networks and institutions that participate in TR, as some of the contributions discussed in our introductory remarks have done, we opted in this paper to focus on the epistemic and material objects of TR (TNBC, BLBC) and their conceptual and technical, biological and clinical dualities. From this point of view, any discussion of TNBC that attempted to draw a line between fundamental and clinically or commercially applied aspects will fail to capture the peculiar dynamics of TR in this domain, insofar as application was built-in from the very beginning in the bio-clinical entities that engendered this domain. If the non-linear view of TNBC research advanced here is correct, then the very idea of research travelling down a metaphorical translational pipeline must be fundamentally flawed. In this regard, our argument is consistent with recent criticism of the notion of a pipeline of drug development. Indeed, as close observers of the drug industry have noted, drug development and TR now both work in a different key: “those who are intimately involved in drug development use network models and management tools with parallel, iterative and self-learning components to orchestrate their projects. Successful drug development in the networked information age requires teams of basic and translational scientists; clinical services; policy, regulation and reimbursement specialists; and consumers, patients and advocates” (Baxter et al., 2013, p.1). In short, it requires the assembling of a new regimen (Cambrosio et al., 2014) of TR. Unlike the “seamless web” favored by historians of technology (Hughes, 1986), and similar to the assemblages analyzed by DeLanda (2006),8 such a regimen is characterized by relations of exteriority, insofar as over time components of TNBC shifted in and out of the assemblage. It also made room for the emergence of novelty, as new instantiations of TNBC on different platforms retroactively affected its more traditional components.

Finally, taking a somewhat broader perspective, TNBC provides us with an indication of the extent to which “the boundaries between biology and medicine have also entered a process of becoming profoundly reconfigured” at the turn of the new century (Rheinberger, 2009). TNBC is intimately linked to the rise of molecular oncology, and, more generally, to the changing configuration of the life sciences. This reconfiguration did not of course happen all at once. As mentioned in the introduction, historians have documented how in the post-WWII period practitioners built interfaces between molecular biology and the clinic, experimenting, for instance, with clinical material. And yet, we would argue that, as exemplified by the clinical trials involving TNBC, the situation has since evolved in important ways, leading to an amalgamation, so to speak, of the patient’s body in the clinical ward and the experimental setting. Clinical interventions in the age of targeted therapy are simultaneously explorations of the molecular mechanisms of normal and pathological cellular processes, and so one can easily maintain that in oncology clinical researchers treat the patient and conduct an experiment (Nelson et al., 2014). TNBC’s trajectory exemplifies these processes, and it’s likely to continue to do so, as this bio-clinical entity undergoes further transformations.

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8 See also Rheinberger (2009, pp. 7 and 11-12) for a discussion of assemblages in biomedicine.
References


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Figure 1. The fragmentation of breast cancer from a common disease to a growing number of rare diseases. Source: Figure 1 (p. 688) in: Harbeck, N., & Rody, A., Lost in translation? Estrogen receptor status and endocrine responsiveness in breast cancer. *Journal of clinical oncology*, 30, 686–689. Reprinted with permission. © 2012 American Society of Clinical Oncology. All rights reserved.
Figure 2. Comparing histological (font style: regular) and molecular (font style: all caps, bold) subtypes. Source: Figure 6 (p. 67) reprinted from *New Biotechnology*, 29, Portier, B. P., Gruver, A. M., Huba, M. A., Minca, E. C., Cheah, A. L., Wang, Z., & Tubbs R. R., *From morphologic to molecular: Established and emerging molecular diagnostics for breast carcinoma*, 665–681, Copyright 2012, with permission from Elsevier.
Figure 3. The intersection between TNBC, BLBC, and BRCA-associated breast cancer, circa 2010. Source: Figure 1 (p. 686), reprinted by permission from Macmillan Publishers Ltd: NATURE REVIEWS CLINICAL ONCOLOGY, Carey, L. A., Winer, E., Viale, G., Cameron, D., & Gianni, L. Triple-negative breast cancer: Disease entity or title of convenience?, 7, 683-692, copyright 2010.
Figure 4. Number of PubMed references from the “breast neoplasms” MeSH subset containing the terms “triple-negative” and “basal-like” in their titles or abstracts.
Figure 5. A “proposed algorithm of stratification” of TNBC. Source: Figure 8 (p. 131), reproduced with permission of ALPHAMED PRESS from Molecular characterization of basal-like and non-basal like triple negative breast cancer, Prat, A., Adamo, B., Cheang, M.C.U., Anders, C.K., Carey L.A., & Perou, C.M. The Oncologist, 18, 123-133; copyright 2013; permission conveyed through Copyright Clearance Center, Inc.