

Chapter 2

Antibody–Drug Conjugate Target Selection: Critical Factors

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Abstract

ADC success requires that all three components of the agent function in a near-flawless manner. Equally important is that the target be selected with stringent consideration as the target is the one factor in ADC development that is immutable and beyond the reach of the developer to refine/manipulate. This chapter reviews the critical factors of target selection that must be met if one is to succeed at ADC development.

Key words Antibody specificity, Antigen expression, Antigen internalization

1 Introduction

The concept of antibody-targeted drugs is not a new one, yet only now is this concept reaching fruition. The long gestational period, marked by many failures, is testimony to the complexity and difficulty of translating this appealingly simple and straightforward theory into practice. The lessons learned by past failures include the realization that any weakness or flaw in any one of the components—antibody, linker, or drug—spells failure for this multicomponent therapeutic agent. And while the term “antibody–drug conjugate” highlights the three component parts of the agent, it ignores the fourth and equally important component in the equation: the target. Indeed, it should be recognized and appreciated that among the four components necessary to yield a successful ADC, only the target is immutable. That is, one may refine and “tweak” the antibody for its affinity, immunogenicity, structure, etc.; the linker for its variable chemistry, cleavability, etc.; and the drug for its potency and mechanism of action, etc., but the target is determined and controlled by nature. It is beyond the drug developers’ ability to tweak, and therefore, its selection must be carefully considered, for if one selects an inappropriate target, no matter how much time, effort, money, etc. are expended on the antibody, drug, and/or linker, the project is doomed to fail.

In this chapter, I will outline some of the most critical elements to be considered in target selection and provide an example of a target to demonstrate how it meets these requirements.

2 Critical Factors in Target Selection

2.1 Specificity

Specificity of the target is the core principle of the ADC approach. Indeed, ADCs are predicated on the principle of targeting a tumor-restricted antigen in order to avoid drug delivery to normal tissues. But tumor specificity must be viewed as a continuous variable rather than a binary one. In practice, one looks for a target with a high degree of tumor specificity where the normal tissue expression is limited in scope and/or present on expendable tissues or tissues with regenerative capacity.

In cases where the target is expressed by normal tissues, over-expression by tumor cells relative to target-positive normal tissue is a critical benefit or requirement. In addition, the accessibility of the tumor cells relative to antigen-positive normal tissue sites, as they relate to ADC biodistribution, is an important consideration. This latter point will be discussed further as well as illustrated in the example described below.

2.2 Level of Expression

Level of expression is critical in several respects. Firstly, it has a significant impact on how much ADC will bind tumor and how much ADC will be internalized into the tumor cell. While a target antigen may be very specific, if it is expressed at a low level, the delta of ADC delivered to tumor versus target-negative normal cells (via nonspecific uptake mechanisms) will be low resulting in a low therapeutic ratio. Conversely a highly expressed target can lead to uptake of cytotoxic doses of ADC and tumor death, while normal tissues that are target antigen-negative or express substantially lower amounts of target may be unaffected. Low expression level of the target, even if highly or absolutely tumor specific, simply may not enable accumulation of adequately cytotoxic doses before non-specific toxicity becomes manifest.

2.3 Internalization

Internalization is important for efficient cytotoxicity when the drug acts intracellularly. Inability of the target antigen to transport the ADC intracellularly severely compromises the efficiency and toxicity of the drug. Targeting an ADC to a non-internalizing target antigen with the expectation that extracellularly released drug will diffuse into the target cell is not a recipe for a successful ADC. While some externally released drug may, indeed, diffuse into the tumor cell, much will also diffuse away from the tumor resulting in compromised efficacy. Ideally, not only is the target antigen internalized but a rapid internalization process combined with efficient recycling or replenishment of antigen at the surface to act as a

virtual pump accumulating intracellular ADC adds to the likelihood of success. One exception to the “rule” of ADC internalization is the case of ADCs armed with radioisotopes where emissions can generally reach the DNA target from the exterior of the cell. Similarly, development of agents that act extracellularly would abrogate the requirement for internalization.

2.4 Target Heterogeneity

Target heterogeneity can be viewed at the level of the tumor type as well as the level of the individual patient. In the former case, I’m referring to the proportion of cases of a given tumor type that are target antigen-positive. Consider the example of the her2 target in breast cancer where approximately 20 % of patients’ tumors are her2 positive. This factor imposes limits on the patient population able to benefit from the therapy as well as a requirement for a companion diagnostic to identify the appropriate patient for treatment.

At the level of intra-patient target antigen heterogeneity, the presence of target-negative tumor cells requires a means to treat those target-negative cells that will not bind/internalize the ADC. This might dictate, for example, the use of a linker that permits release of drug from target-positive cells to reach nearby target-negative cells (so-called bystander effect). But, clearly, the greater the proportion of target-negative cells, the more tenuous the ADC target is.

2.5 Accessibility

Tumor/target accessibility is another critical factor. With respect to this issue, solid tumors represent a higher hurdle than hematogenous (or “liquid”) tumors. The latter are present in blood, bone marrow, and/or lymph nodes—sites that receive high levels of circulating ADC. Conversely, it has been amply demonstrated that solid tumors pose difficulty to the penetration of drugs, ADCs, or otherwise (including small molecules) [1, 2]. The bulkier the tumor, the more necrotic and the greater the difficulty of the ADC to reach its tumor target no matter how specific or highly expressed it is.

3 Relative Factors/Considerations in Target Selection

3.1 Identifying the Appropriate Patient Population

It is fundamentally important, and becoming increasingly more practically important, in any cancer setting and with any oncologic agent, that the optimal patient population be prospectively identifiable. Regardless of the ADC target, there are likely to be patients whose tumors are target antigen negative. Her2, targeted by trastuzumab-DM1 (T-DM1), provides an excellent example where 75–85 % of breast cancer patients are her2-negative. In the absence of a means to identify appropriate patients, it would be virtually impossible to develop an acceptable therapeutic. Identification of the target-pos population is critical for the success of the therapy.

This is also true in cases where the target is expressed at widely varying levels; in such a case, it would be beneficial to identify patients whose tumors express target levels above a threshold that can be defined in early clinical trials.

3.2 Target Antigen Modulation

With some targets, more commonly described in lymphoid cells, antibody binding may lead to depletion of the target antigen for a period of time after Ab binding (“antigenic modulation”). In such cases, it will be imperative to know the kinetics of target modulation and re-expression in order to optimize dosing. It will also be important to identify if/when selection of target-negative cells occurs as a result of selection pressure from the ADC treatment. Although less likely, in some cases, it may be possible to upregulate the target. An example of the latter phenomenon will be discussed below.

4 Case Study: Prostate-Specific Membrane Antigen

Having enumerated key features of target selection, the following case study of prostate-specific membrane antigen (PSMA) presents an exemplary ADC target. I will review each of the features outlined above to demonstrate what makes PSMA a prototypical ADC target.

4.1 Specificity

Initial studies of PSMA [3, 4] reported its near-absolute prostate specificity and led to the proposed designation “prostate-specific membrane antigen.” Subsequent studies have shown that PSMA is expressed by renal proximal tubules and some astrocytes and more weakly by small bowel and salivary gland (Fig. 1). As a result, PSMA resembles other highly restricted differentiation antigens that often serve as cancer targets. In general, these antigenic targets are about as tumor specific as one can get in the ADC field.

Another consideration in the realm of specificity is whether target-positive normal tissue subject to ADC binding is necessary to support/maintain life and/or whether the tissue can regenerate. Clearly, in the case of PSMA, if renal or bowel toxicity were to occur, it would be problematic; conversely, prostatic loss may not be a problem (except perhaps where fertility was important). In other cases, loss of normal cells can be tolerated particularly if they subsequently repopulate as in the case of CD20-pos normal lymphocytes.

But there is another consideration alluded to in the discussion above that is of practical importance: the precise cellular site of expression may offer some additional specificity benefit. In the case of PSMA, for example, potential ADC targeting of astrocytes is prevented by the blood–brain barrier. And in normal tissues such as the prostate, kidney, and small bowel, PSMA is expressed in

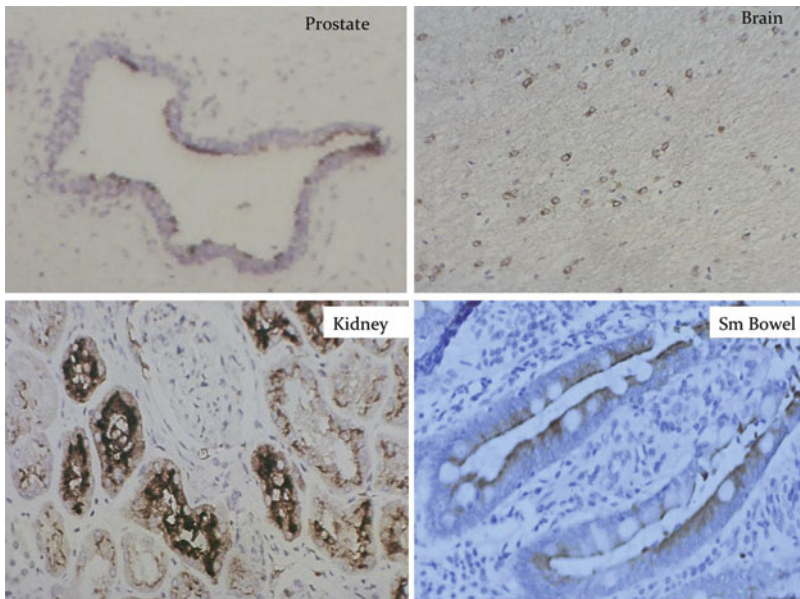


Fig. 1 Immunohistochemical demonstration of normal tissue expression of PSMA. Note that in the epithelial sites (prostate, kidney, bowel), expression is polarized and limited to the apical/luminal border

a polarized manner at the apical/luminal aspect of the cell (Fig. 1). These normal tissue sites are effectively *ex vivo*, and antibody access is prevented by intervening cell layers and basement membrane and by tight junctions. Based on human data of concentration gradients across such barriers, we estimate that these normal tissue sites achieve Ab concentrations of approximately 10^{-7} that of plasma.

Perhaps the lesson is that absolute tumor specificity is not a prerequisite, but a high degree of specificity is important, and there are additional considerations such as those described that can mitigate toxicity even when target expression occurs on critical normal tissues.

4.2 Level of Expression

PSMA is significantly upregulated in prostate cancer (PC) relative to its expression in other tissues including normal prostate (Fig. 2). In human PC cell lines, LNCaP, C4-2, and MDA-PCa2b express levels measured in the millions of molecules per cell [5]. Sokoloff et al. [6] used human tissue specimens and showed that PC expresses some 100- to 1,000-fold higher levels than any normal tissues.

4.3 Internalization

PSMA is rapidly and efficiently internalized [7] by an endocytic pathway (Fig. 3). In addition, it rapidly recycles back to the membrane therefore virtually pumping Ab payload (ADC) into the cell. Between high level expression and efficient internalization, high amounts of payload are efficiently internalized.

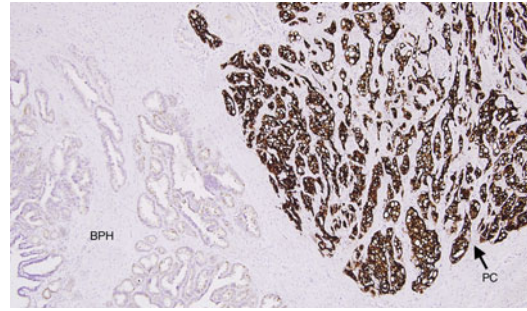


Fig. 2 Prostate cancer significantly upregulates PSMA expression relative to normal or benign prostatic hyperplasia (BPH). Note also the homogeneity of PSMA expression within the cancer

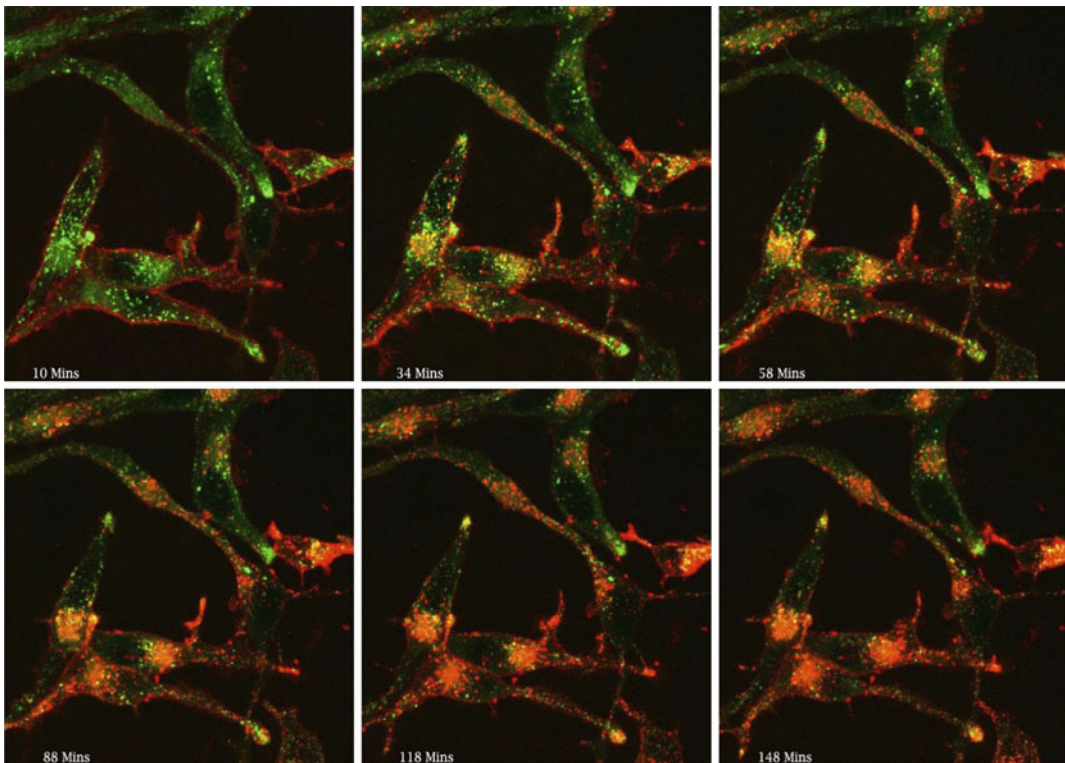


Fig. 3 Time-lapse confocal video-microscopy demonstrating internalization of J591, an anti-PSMA antibody, into viable prostate cancer cells (LNCaP). J591 is directly labeled with alexa-647 (*red*) dye; lysosomes are labeled green with LysoTracker[®] (Invitrogen). Incubation takes place at 4 °C and the clock starts when the cells are moved to 37 °C. Initially (10 min), the J591 (*red*) can be seen bound to the plasma membrane. Over time, an increasing amount of J591 Ab can be seen accumulating in a lysosomal compartment (*red + green* = *orange*) adjacent to the nucleus

4.4 Heterogeneity

As discussed above, heterogeneity should be considered at the inter-patient and the intra-patient levels. At the inter-patient level, in the case of PSMA, studies have shown that approximately 95 % of PC are PSMA positive [8–16] thus making a very high proportion

of PC patients potential treatment candidates. At the intra-patient level, the literature suggests that patients with advanced, metastatic, castrate-resistant PC express high levels of PSMA in a rather homogeneous manner (*see* Fig. 2). Again, this is a favorable property for an ADC allowing greater cytotoxic efficacy.

4.5 Accessibility

Hematopoietic/liquid tumors are the most accessible to circulating ADC where the tumor cells are effectively bathed in ADC-containing plasma. It is no coincidence that the two first ADCs attaining FDA approval were CD33-pos AML (gemtuzumab ozogamicin) and CD30-pos lymphomas (brentuximab vedotin). Solid tumors present a significantly higher hurdle, but there are exceptions. For example, breast cancer, where T-DM1 is approaching regulatory approval, is a cancer that spreads predominately to lymph nodes and bone marrow—these are sites that “see” very high levels of circulating Ab, approaching those seen in plasma, due to their more porous endothelial junctions. Indeed, the same is true of PC, where the most common site of spread is bone marrow (85–90 % of patients) followed by lymph nodal disease (in 20–50 % of patients). In addition, the availability of a widely used biomarker in PC, prostate-specific antigen (PSA), provides a lead-time warning of tumor recurrence (which occurs in approximately 30 % of patients who undergo local treatment) of several years before visible on imaging studies. At the time of PSA elevation, the tumor burden is measurable in grams, substantially lower than at the time of imagable recurrence of other solid tumor types. This allows initiation of treatment at the time of a very small tumor burden when metastases are composed of small clusters of cells primarily in bone marrow (Fig. 4). As a result, ADC penetration of

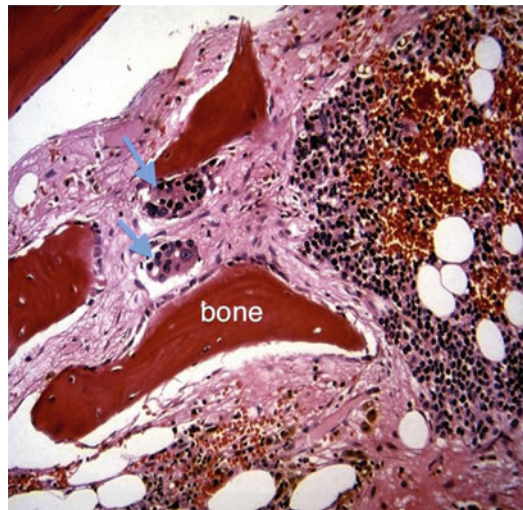


Fig. 4 A bone marrow biopsy showing two small islands of PC metastases (*arrows*) adjacent to bone spicules. These small islands of tumor cells would be very accessible to circulating Ab/ADC

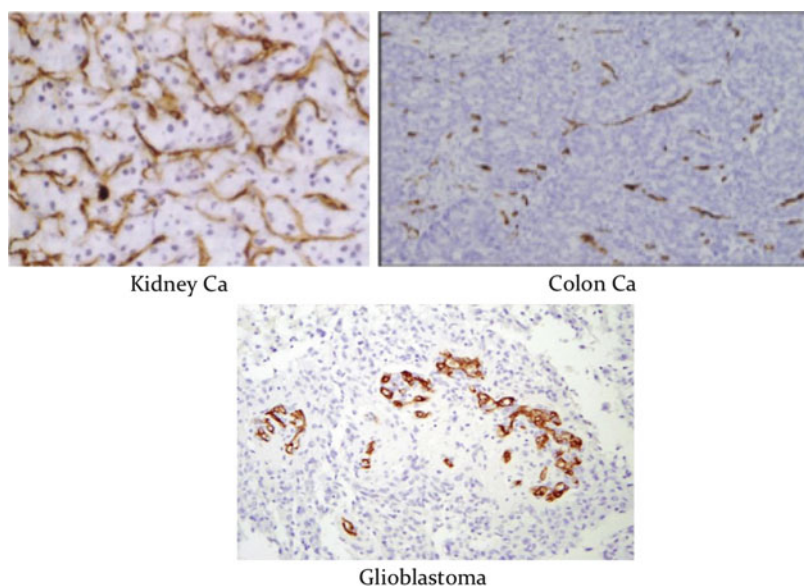


Fig. 5 Examples of three different solid tumor types demonstrating PSMA expression (*stained brown*) by neovascular endothelium

tumor in this setting approaches that of hematopoietic tumors and exceeds that of any other solid tumor. As a result, at the macro level, i.e., sites of disease, and at a micro level—tumor burden and antigen target accessibility—PC represents a very favorable target.

Another therapeutic opportunity for the PSMA target relates to its expression on the neo-vasculature of virtually all types of solid tumor, but not by normal vasculature [17–21] (Fig. 5). Expression is on the endothelial surface exposed to the circulation, and based on immunohistochemical comparisons, expression levels are in a similar range to that seen in PC. Obviously, neovascular PSMA would be highly accessible to an ADC.

4.6 Identifying the Appropriate Patient Population

We discussed above the importance of being able to identify appropriate patients and gave the example of her2-targeted therapies. In the case of PSMA, we have previously mentioned that 95 % of PC are PSMA positive. Indeed, when we began using mAbs to PSMA to target disease in clinical trials, we considered any PC patient who met the clinical criteria to be eligible. While the immunopathological data from multiple labs [8–16] covering almost 1,500 patients does, indeed, support this point, we have more recently come to appreciate that there are significantly different levels of PSMA expression among patients even when at the same state of disease [22]. Our planar imaging studies done in well >100 patients show that we can target PC, virtually flawlessly, in 90–95 % of patients (Fig. 6), consistent with the immunopathological data.

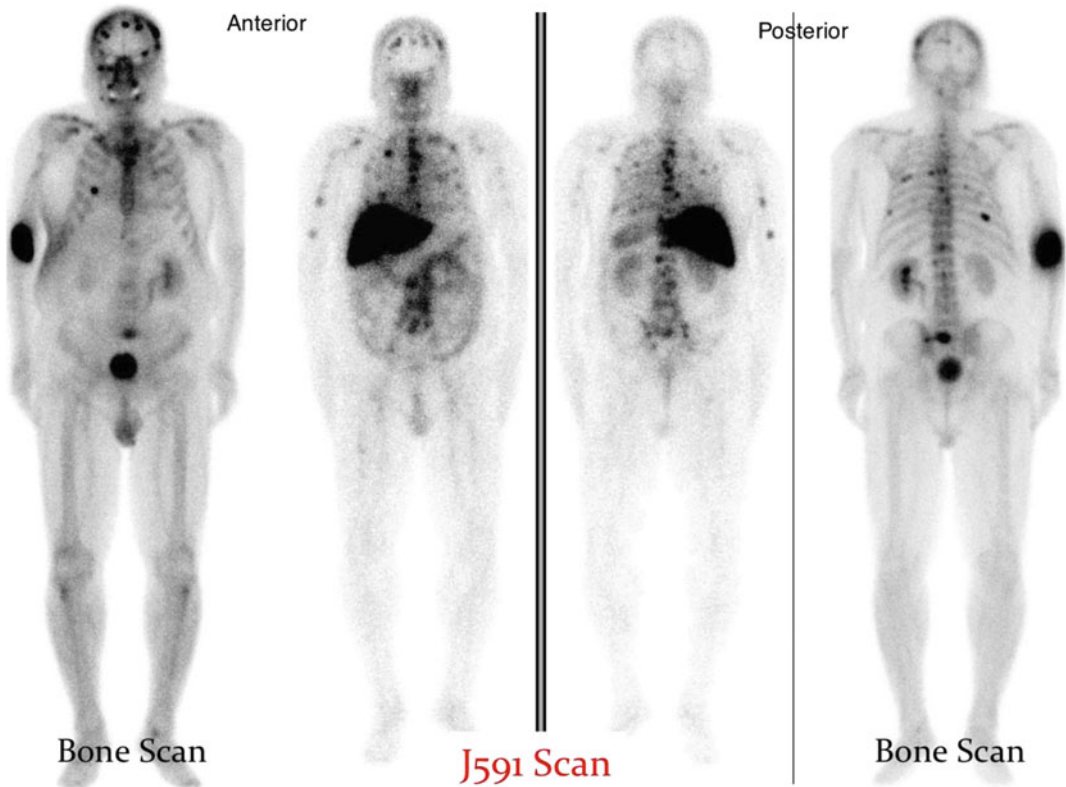


Fig. 6 Representative planar images of a patient with PC. Far *left* and far *right panels* represent anterior and posterior ^{99m}Tc methylene diphosphonate (MDP) bone scan images, respectively. *Center panels* show radiolabeled J591 mAb anterior and posterior images allowing direct comparison to bone scan. In a bone scan, the radioisotope is excreted by the urinary tract (note obstructed left kidney and bladder); in the Ab scan, the radiometal is excreted by the liver. Also apparent in the bone scan is right antecubital fossa extravasation of the isotope at the injection site. Note that every bone lesion seen on the bone scan is visible on the J591 Ab scan. In addition, the Ab scan picks up many more (true positive) lesions than the bone scan. The *midline* abdominal uptake on the anterior J591 Ab image represents retroperitoneal nodal disease that obstructed the *left* kidney

But these images, while nonquantitative, suggest that there is a wide range of expression level. Our preliminary impression suggests that there is a correlation between the image intensity (corresponding to expression level) and the likelihood of response. As a result, as our clinical trials are maturing, we have begun incorporating studies to define the small group of PC patients who are PSMA negative and exclude them, and we also plan to use quantitative analyses, either PSMA PET imaging or analysis of circulating tumor cells to define the patient subset with the highest level of expression who would be the best candidates for a PSMA ADC. Certainly, we'd anticipate that similar approaches can and will be brought to bear with other targets, and we are aware, for

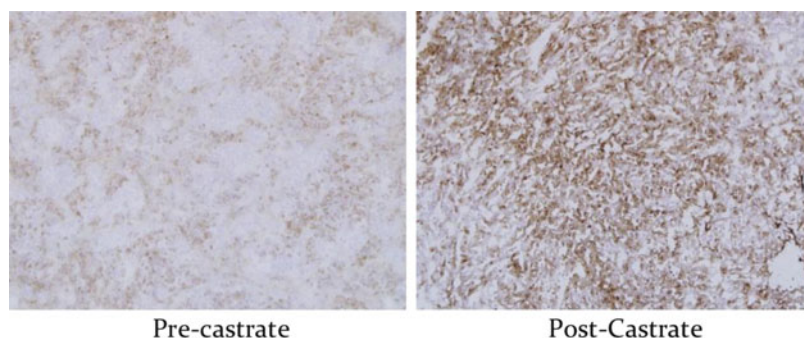


Fig. 7 Example of PC cell line (CWR22Rv1) that expresses low levels of PSMA in a non-castrate animal. After castration, PSMA expression increases considerably as demonstrated by immunohistochemistry. In vitro, this cell line increases PSMA expression by four- to fivefold after castration. The increase can also be detected by J591 PET imaging [25]

example, that effort is underway to develop PET imaging of her2 [23] which may become clinically useful in this setting as well to further improve patient selection.

In the case of neovascular expression, we have noted that PSMA is expressed by virtually every type of solid tumor yet the proportion of cases that are PSMA positive vary from one tumor type to another [22]. In addition, we have also found that the intensity of expression can also vary, again suggesting that ways to identify optimal patients will be very valuable.

4.7 Can Target Expression Be Modulated

An interesting property of PSMA is that its expression is androgen-regulated; when patients are placed on hormonal therapy, it induces upregulation of PSMA. In vitro data suggests [24] that PSMA can be upregulated by as much as 80-fold. We have also found that this upregulation occurs even in so-called “castrate-resistant” or “androgen-insensitive” PC models (Fig. 7). Using xenograft models of such a castrate-resistant tumor, we have shown that one can increase antitumor efficacy of an ADC [24]. Fortuitously, not only are antiandrogenic agents approved for use, but they also represent the cornerstone of treatment for this disease. In addition to directly enhancing tumor efficacy, this approach also improves the therapeutic window as modulating the androgen receptor (AR) and upregulation of the PSMA target occurs only in the AR-positive PC cells and not the normal tissues that express PSMA but are AR negative. This phenomenon of target upregulation, which we have termed “conditionally enhanced vulnerability/sensitivity,” may be feasible in other target/tumor types. One can easily set up screens to potentially identify agents capable of upregulating the target of interest.

5 Conclusions

There is little doubt that weaving together the necessary components of an ADC requires optimal execution with respect to all of the components of the agent—antibody, linker, and drug. But it also requires a target that must meet equally stringent criteria, and the target is subject to few if any manipulations by the drug developer. Based on the criteria outlined above, it is likely that there are a relatively small number of tumor targets that would be optimal for ADC targeting. Moreover, with the requirement for high level expression in order for current cytotoxic agents to be effective, it is likely that most good targets have already been identified. Development of innovative ways to identify novel, specific, but lower level expressed targets is unlikely to yield many new targets unless more potent cytotoxics are developed, and this in turn would put further constraints of the performance of the linker.

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