



# Secondary Antibody-Drug Conjugates As Tools For ADC Discovery

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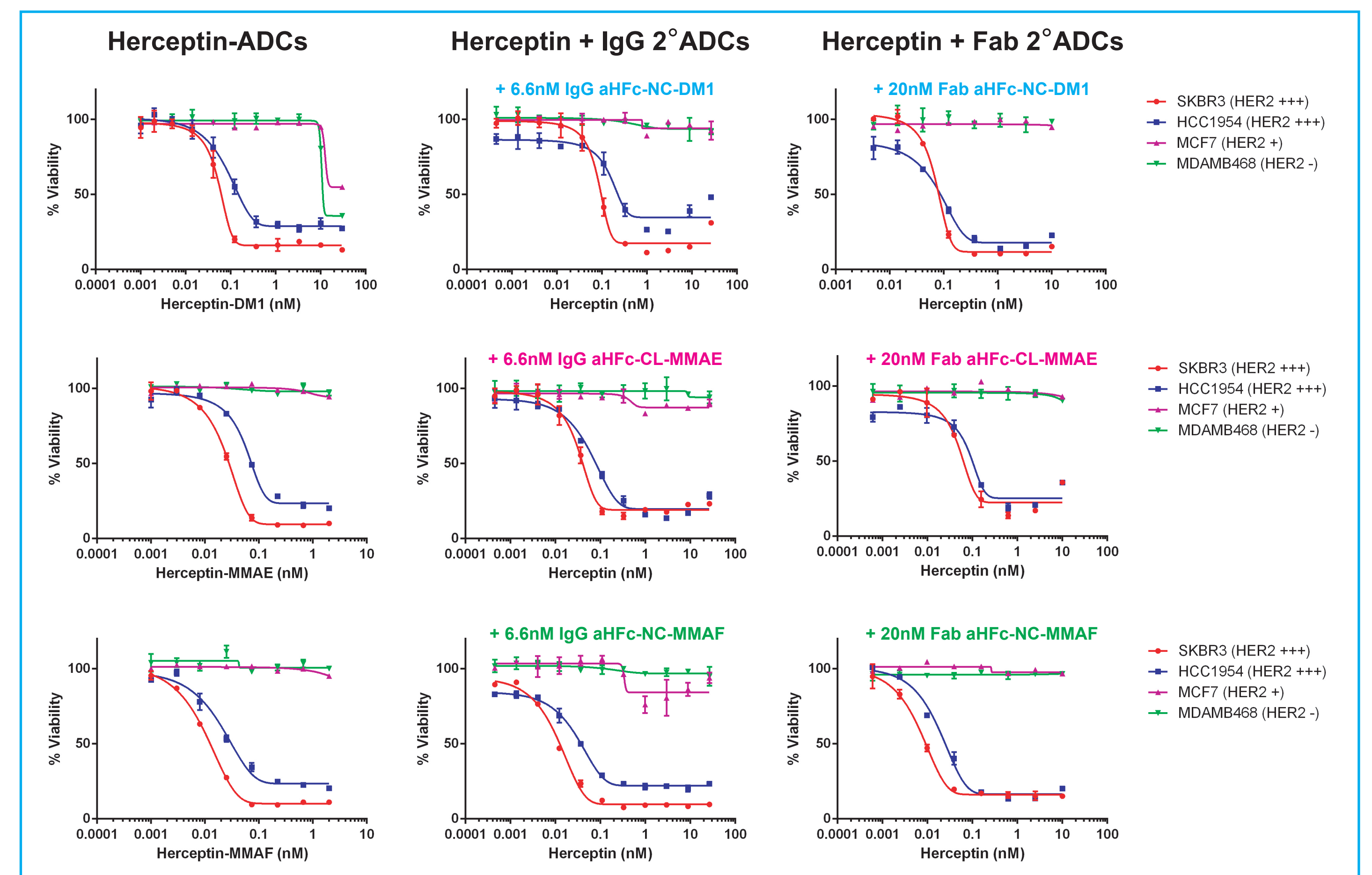
## ABSTRACT

Currently many companies and laboratories have initiated antibody-drug conjugate (ADC) discovery programs aiming to develop better anti-cancer therapeutics. To facilitate more cost effective early phase ADC research and attract more scientists into doing ADC studies, Moradec has produced multiple panels of secondary antibody-drug conjugates (2°ADCs) linked to state-of-art toxins such as MMAE, MMAF, DM1, or Duocarmycin, etc. Cell-based assays using unconjugated primary mAbs in the presence of 2°ADCs are quick, relevant, and economical alternatives to direct conjugates for pre-screening the mAbs as ADC candidates and validating relevant receptors for ADC targeting. Here we compare the cell killing profiles of three Herceptin-ADCs to those of naked Herceptin in the presence of 2°ADCs. We demonstrate both full-length IgG and Fab fragment format 2°ADCs are valid and useful tools in ADC proof-of-concept studies or high-throughput screening.

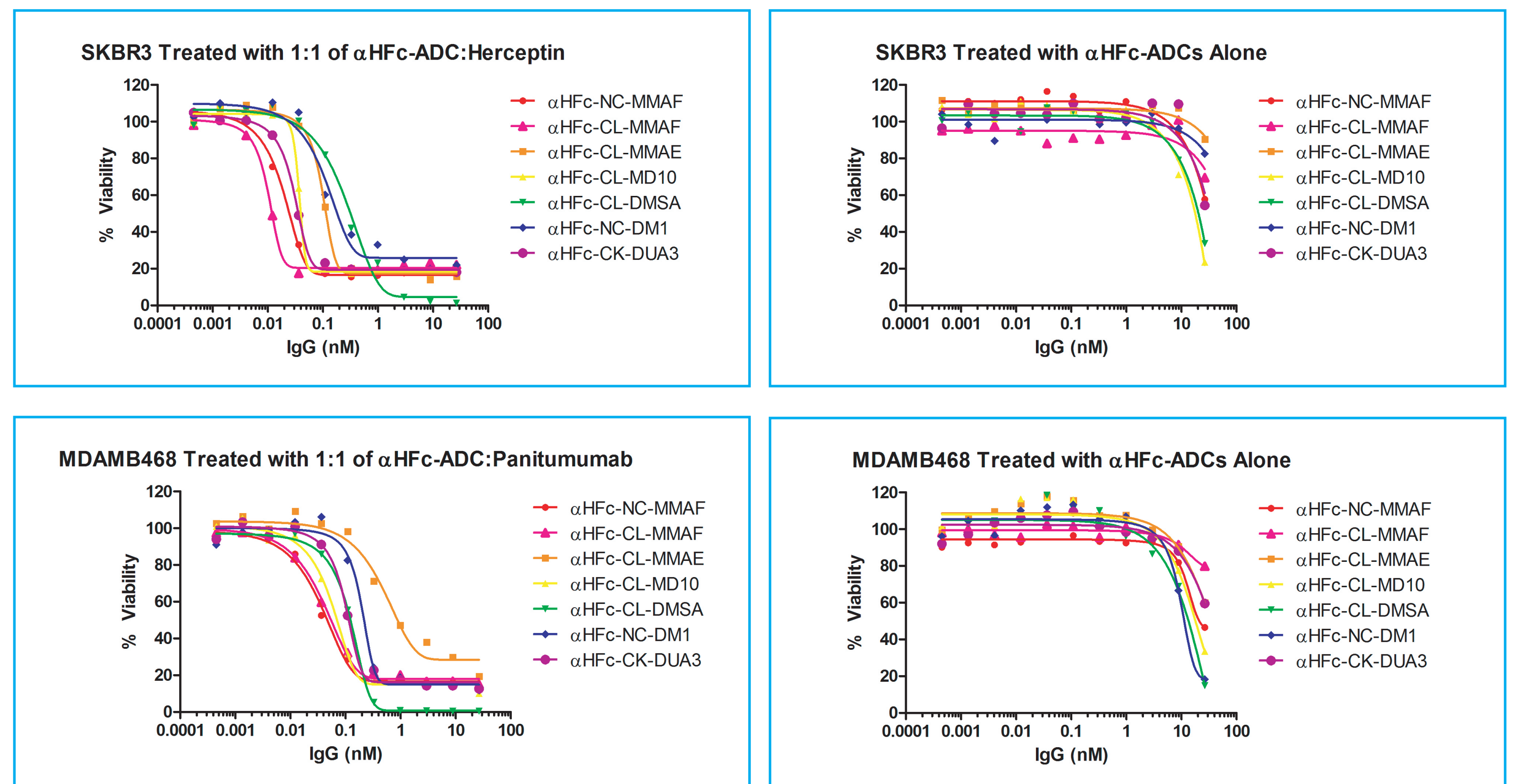
## INTRODUCTION

To most biologists who intend to test the function of ADCs in cell-based assays, conjugating mAbs with toxins is a daunting, time consuming and expensive task. Direct conjugation requires not only obtaining milligram quantities of purified antibodies, separate conjugation of each antibody, and further isolation of the ADC from the unconjugated drug, but also handling highly toxic compounds. This challenge can be overcome by applying the 2°ADC approach. Instead of using primary ADCs, cell-based assays are carried out using unconjugated mAbs in the presence of a 2°ADC. Microgram quantities of unconjugated primary mAbs (without purification) are needed for an experiment, and cell killing results can be obtained as few as two to three days. The 2°ADC approach is a quick and economical alternative to pre-screening mAbs as ADC candidates against target cells.

Using Herceptin targeting Her2-overexpressing tumors cells as an example, we compared the cytotoxic profiles of Herceptin direct ADCs to those of unconjugated Herceptin in the presence of corresponding 2°ADCs. SKBR3 and HCC1954 are Her2 overexpressing cells, MCF7 has normal Her2 expression, and MDA-MB468 is Her2 negative. All three direct ADCs (Herceptin-DM1, Herceptin-MMAE, and Herceptin-MMAF) have potent cytotoxicity against Her2-overexpressing SKBR3 and HCC1954 cells, while show little toxicity on Her2 normal MCF7 or Her2 negative MDA-MB468 cells (left panels). Similar results can also be achieved using naked Herceptin in the presence of corresponding IgG or Fab format 2°ADCs (middle and left panels, respectively). The cell killings in the 2°ADC assays are Herceptin-dependent and Her2-specific.



Moradec has a variety of  $\alpha$ HFc-ADCs with cleavable (CL) or non-cleavable (NC) linker to different cytotoxic drugs, such as MMAE, MMAF, MD10, DM-1, DUA3, and DMSA, etc, which are specific and effective screening tools. The anti-human Fc ADCs ( $\alpha$ HFc-ADCs) are ideal for pre-screening antibodies with a human IgG Fc moiety or recombinant proteins with human IgG Fc fusion to determine their cytotoxicity. For instance, the  $\alpha$ HFc-ADCs display specific and potent killing of the Her2 overexpressing SKBR3 in combination with naked Herceptin, while show little killing in the absence of Herceptin. Similarly, the  $\alpha$ HFc-ADCs display specific and potent cytotoxicity against the EGFR overexpressing MDAMB468 in the presence of EGFR-specific Panitumumab, while show little killing alone. When applied in combination with tumor specific monoclonal antibodies or recombinant Fc fusion proteins, the  $\alpha$ HFc-ADCs can help determine the cytotoxic potential for these antibodies or proteins against target cell lines. Simultaneously testing the efficacy of multiple drug-linker combinations for your specific cell lines helps you to make better decision on the final format of your antibody-drug conjugate.



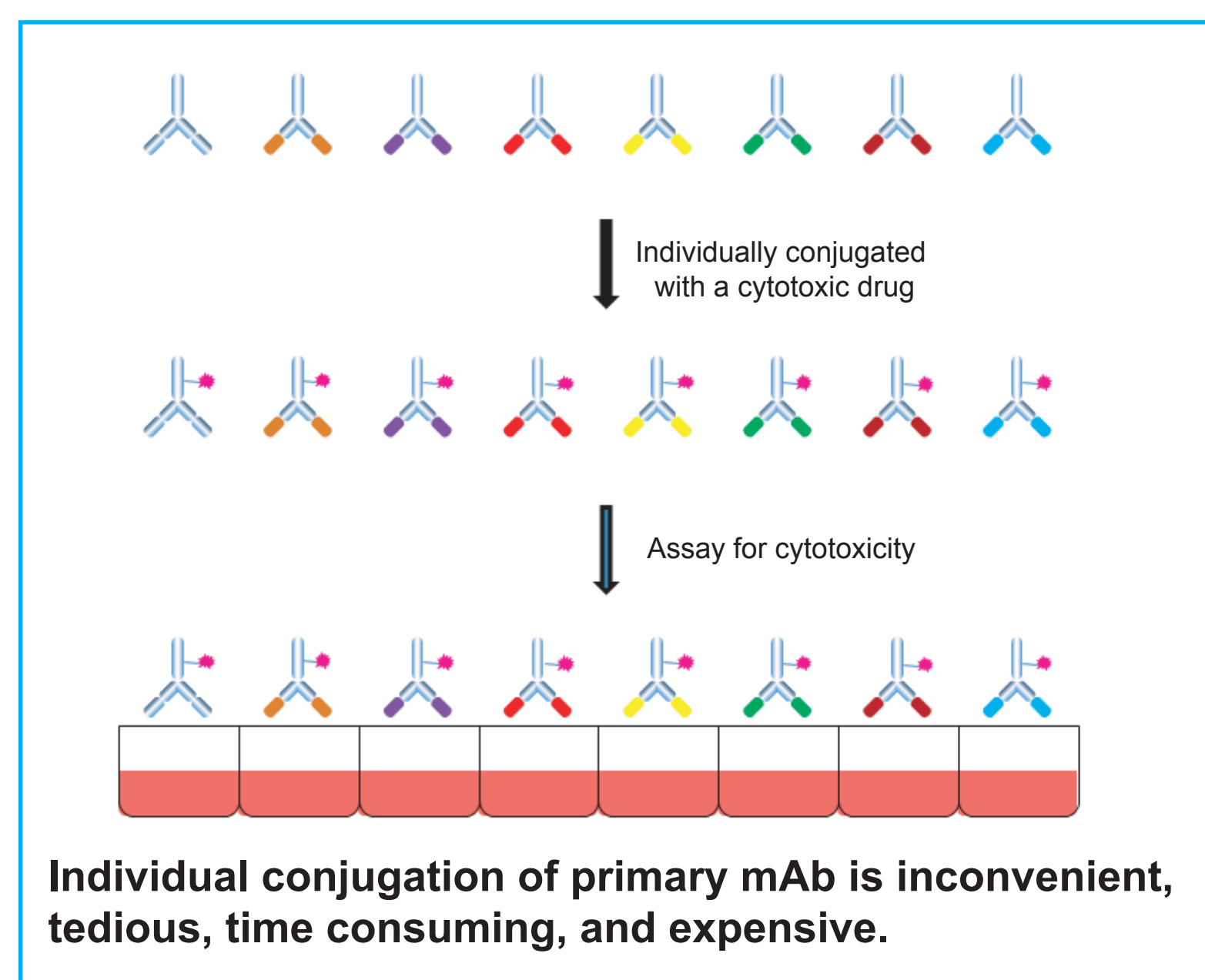
In addition to anti-human 2°ADCs, Moradec also provides anti-mouse, anti-rabbit, and anti-rat 2°ADCs. These 2°ADCs are ideal for carrying out initial proof-of-concept studies of tumor targeting using commercial mAbs. As few as several micrograms of mAbs are need to do a cytotoxic experiment and get the answers. Once a positive answer is confirmed, more resource can be put into place to humanize and/or affinity mature the antibodies. This attribute is particularly attractive for those labs without any significant antibody library resources. Furthermore, the 2°ADCs are also useful in high-throughput screening of antibodies from hybridoma supernatants. Assays can be carried out using hybridoma supernatant directly without purification because the 2°ADCs are specific to either mouse, rabbit or rat IgGs and have little cross-activities to bovine IgGs.

In choosing a 2°ADC in your cell based assays, the IgG and Fab fragment formats are both valid tools in proof-of-concept studies. Depending on receptors being targeted, the Fab 2°ADCs may provide certain advantages such as minimizing receptor cross-linking effect and potentially reduce the false positive results in cell-based assays. Furthermore, because of their monovalence nature, the Fab 2°ADCs are likely better in ranking the internalization efficiencies of multiple primary mAbs. Nonetheless, cell-based assays using Moradec's 2°ADCs allow you to quickly screen an array of primary antibody leads and identify the best candidate for making specific ADCs.

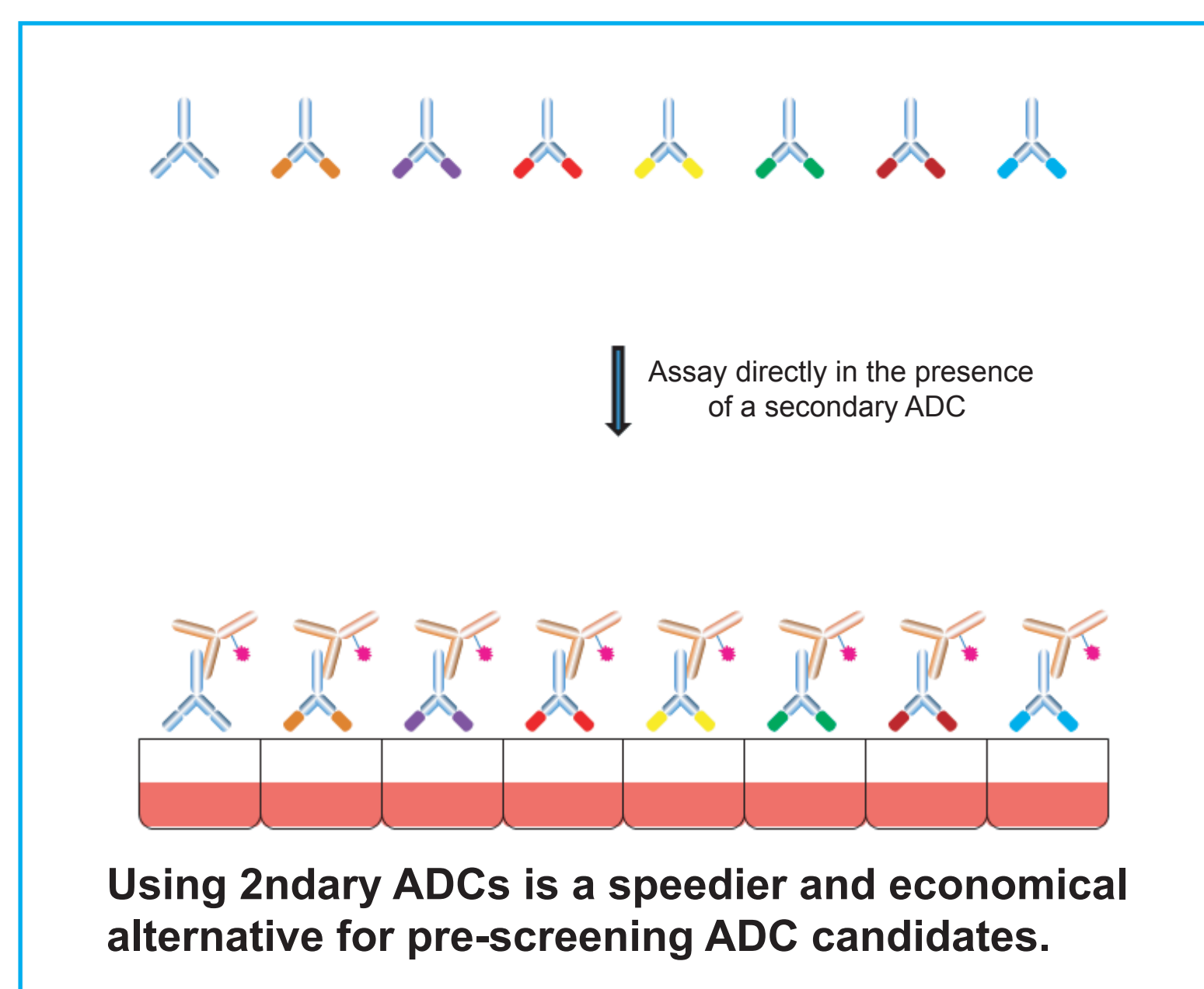
## CONCLUSIONS

Moradec provides state-of-the-art secondary antibody-drug conjugates linked to potent cytotoxic small molecules such as MMAE, MMAF, DM1, or Duocarmycin, etc. These 2°ADCs alone have minimal toxicity while showing specific and potent killing of tumor cells in the presence of appropriate primary antibodies recognizing the overexpressed tumor markers. Instead of laborious and expensive conjugation of individual monoclonal antibodies, using the 2°ADCs in cell based assays is a faster and cheaper approach for prescreening target antibodies against cancer cells. More importantly, the 2°ADCs are conjugated with well sought-after cytotoxic drugs such as potent tubulin inhibitors or DNA damaging molecules. Therefore testing relevant MOAs and efficacy of different drug-linker combinations has become much easier without spending lots of money and time on individual conjugation. Our approach allows you to make better decisions on the final format of your antibody-drug conjugates and be more confident of the MOA tested in the initial proof-of-concept studies. Hence Moradec's 2°ADCs are great tools for your ADC discovery.

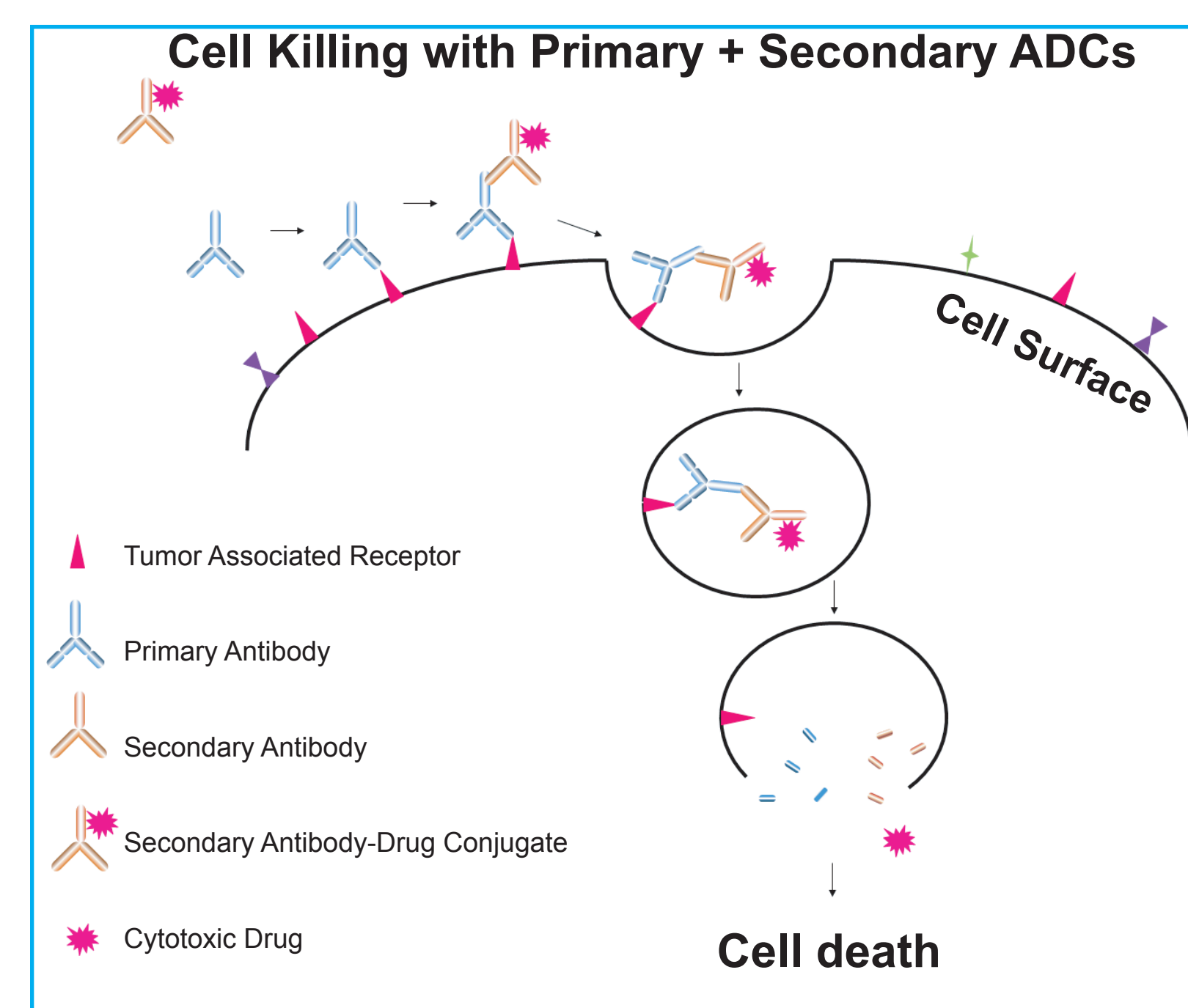
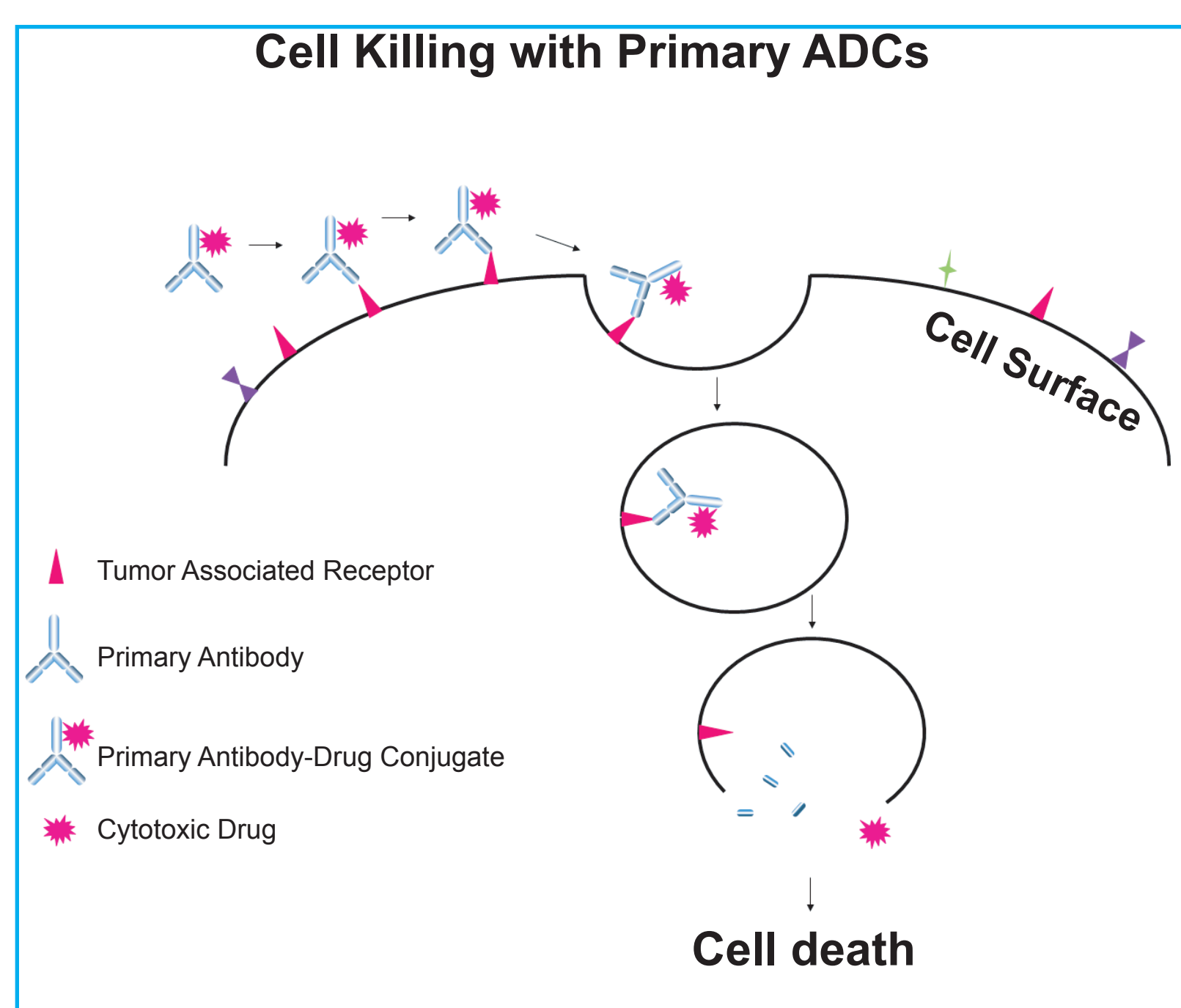
### Traditional Approach



### Moradec's Approach



An ADC functions in cell killing by first binding to a target cell surface receptor and subsequent internalization with the ADC/receptor complex. Releasing and accumulating of sufficient amount of small molecule toxins within the cytoplasm leads to cell death. In theory, target-specific killing can also be accomplished using the unconjugate primary mAb in the presence of a 2°ADC. As long as the 2°ADC does not interfere with the mAb/receptor complex formation, receptor internalization of the mAb/2°ADC complex can also achieve the dose-dependent drug release within the cells similar to that of the primary ADC. In addition, the 2°ADC should have minimal toxicity to the target cells in the absence of the primary mAb.



## RESULTS

Moradec has developed multiple panels of 2°ADCs linked to many highly sought-after cytotoxic small molecules. These ADCs are designed with the following concepts in mind. 1) The secondary antibodies recognize either the Fc or the heavy/light chain portion of human, mouse, rabbit, or rat IgGs, commonly found in the format of many therapeutic antibody discovery programs. 2) The 2°ADC format can be either whole IgG or Fab fragment. 3) The small molecule toxins include the well-known tubulin inhibitors such as Monomethyl Auristatin A (MMAE), Monomethyl Auristatin F (MMAF), Monomethyl Dolastatin 10 (MD10), Dualstatin 3 (DUA3), and Maytansinoids 1 (DM1) and 4 (DM4), plus potent DNA damaging reagents such as Duocarmycin SA (DMSA) and Calicheamicin (CALI). 4) The linkers connecting the secondary antibodies to the small molecule drugs are either non-cleavable or cleavable by endosome cathepsins or free thiol reducing molecules. These 2°ADCs are better suited for targeting tumor cells via either tubulin polymerization inhibition or DNA strand-breaking mechanisms, which are highly sought-after cell killing mechanisms of action (MOAs).

