Secondary Antibody-Drug Conjugates As Tools For ADC Discovery

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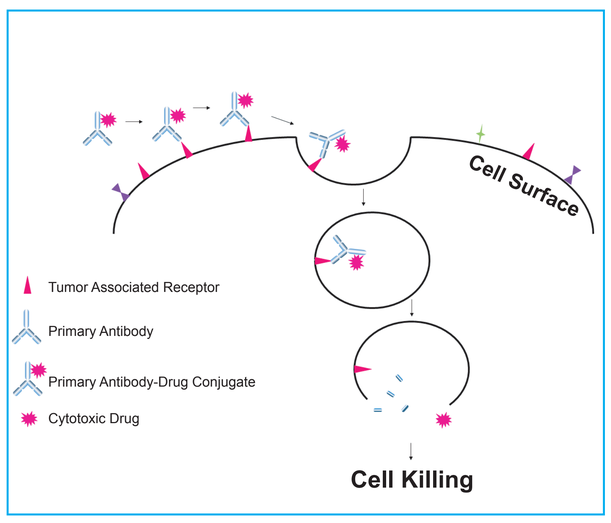
ABSTRACT

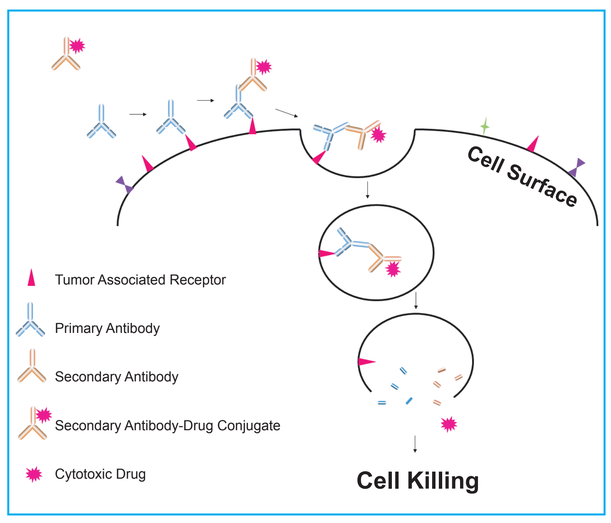
Therapy using antibody-drug conjugates (ADCs) is a promising approach in better targeting cancer cells. Many pharmaceutical and biotechnology companies have initiated programs for ADC discovery. Moradec LLC has developed state-of-the-art secondary antibody-drug conjugates (2°ADCs) as tools for effective, economic, and relevant ADC discovery. These 2°ADCs are conjugated with cleavable or non-cleavable linkers to different cytotoxic small molecules, such as MMAE, MMAF, Dolastatin, DM-1, DM-4, Duocarmycin, and Calicheamicin. Instead of individually conjugating your target mAbs, using the 2°ADCs in a cell-based cytotoxic assay is a quick and economical alternative to pre-screening the mAbs as ADC candidates against cancer cells. In addition, the 2°ADCs are great tools for identifying relevant receptors on cancer cells for ADC targeting.

INTRODUCTION

Antibody-drug conjugates (ADCs), which consist of an antibody linked to a cytotoxic drug, have become a new targeted therapy against cancer. The idea is based on the premise of selective binding of the ADCs to target cancer cells via the monoclonal antibody (mAb) portion and specific release of the drugs to do their damage upon internalization of the ADCs. This achieves target-specific killing of tumor cells while minimizing systemic toxicity of the cytotoxic drug.

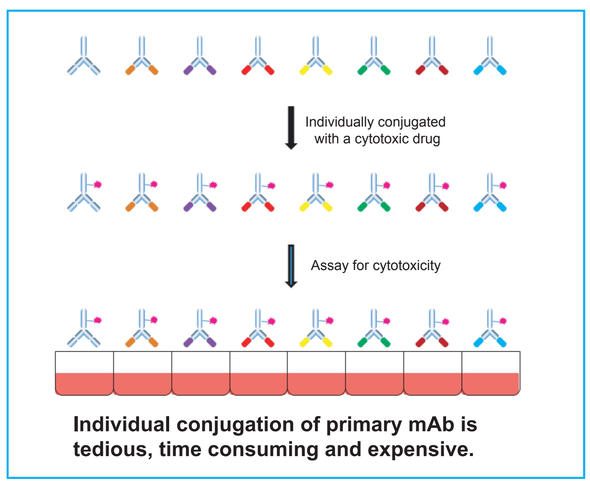
Prior to testing the function of ADCs in cell-based assays, each mAb is typically conjugated with a cytotoxic drug directly. This step is time consuming and expensive, requiring milligram quantities of purified antibodies, separate conjugation of each antibody, and further isolation of the ADC from the unconjugated drug. **Using secondary antibody-drug conjugates (2°ADC) in a cell-based cytotoxic assay is a quick and economical alternative to pre-screening mAbs as ADC candidates against tumor cells.** Instead of conjugating the mAbs individually with a cytotoxic drug, the naked antibody is added directly to the cells in the presence of a 2°ADC. Internalization of the mAb/2°ADC complex can achieve a similar effect of dose-dependent drug release within the cells as that of the direct antibody-drug conjugate, while cells expressing low density of the targeted receptor are not affected.





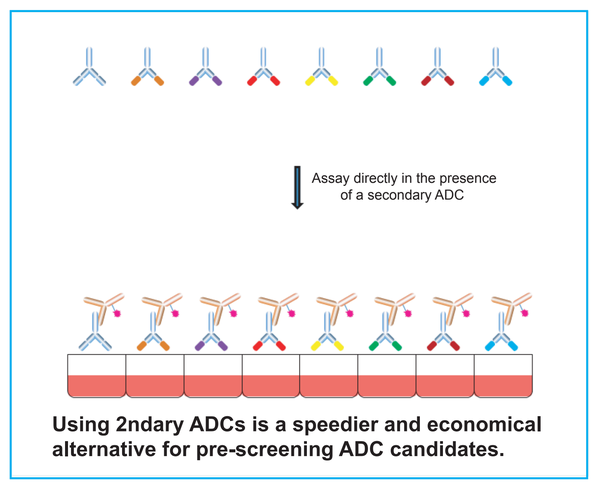
With this principle in mind, Moradec produced a panel of 2°ADCs with either cleavable or non-cleavable linkers to state-of-the-art small molecule cytotoxic drugs. *In vitro* cell-based assays using the 2°ADC/mAb combinations enable rapid screening of an array of primary antibodies in multiple cell lines. Simultaneously testing the efficacy of multiple drug-linker combinations in ADCs facilitates better decision making on the final format of the lead ADC. This is a quicker and more economical way to identify the best candidate for making specific ADCs. Not only can the 2°ADCs be used for screening antibodies, they can also be applied to recombinant protein ligands with Fc fusion for targeting cell-surface receptors.

Traditional Approach



Individual conjugation of primary mAb is tedious, time consuming and expensive.

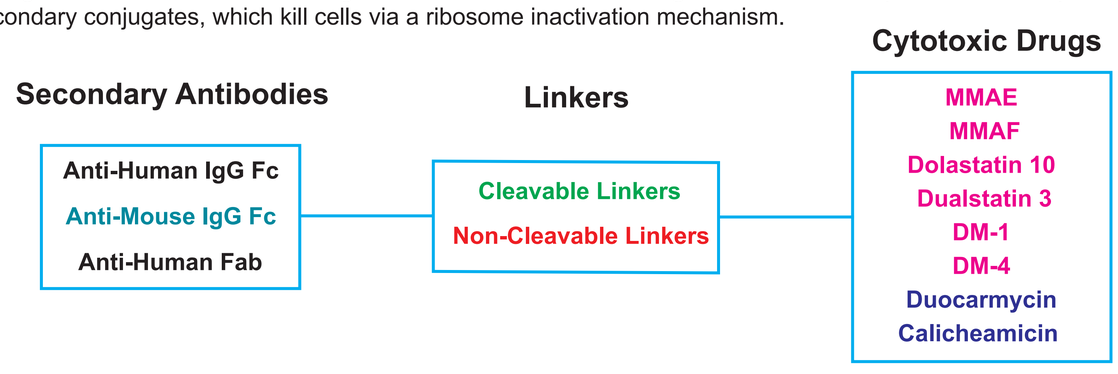
Moradec's Approach



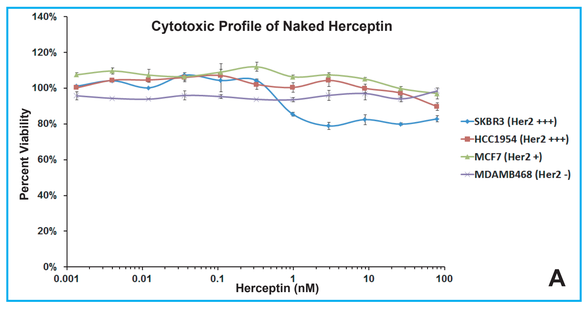
Using 2ndary ADCs is a speedier and economical alternative for pre-screening ADC candidates.

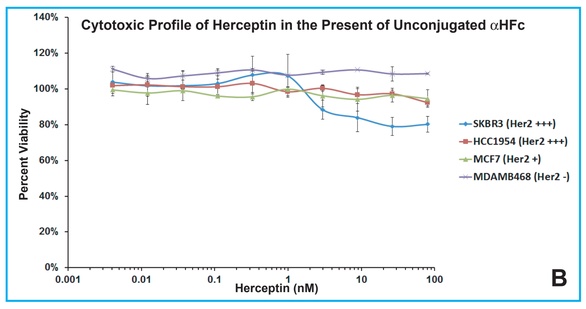
RESULTS

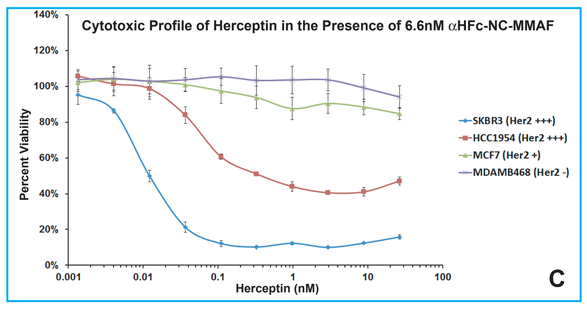
Moradec has developed multiple panels of 2°ADCs linked to many highly sought-after cytotoxic small molecules. These ADCs are designed with the following concepts in mind: **1)** The secondary antibodies recognize either the Fc or Fab portion of human or mouse IgGs typically found in the format of many therapeutic antibody discovery programs. **2)** 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). **3)** 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. Moradec's 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 (MOA). Moradec's ADCs are more appropriate surrogates for cell-based screening because of the relevant drugs and linkers compared to the saporin-based secondary conjugates, which kill cells via a ribosome inactivation mechanism.

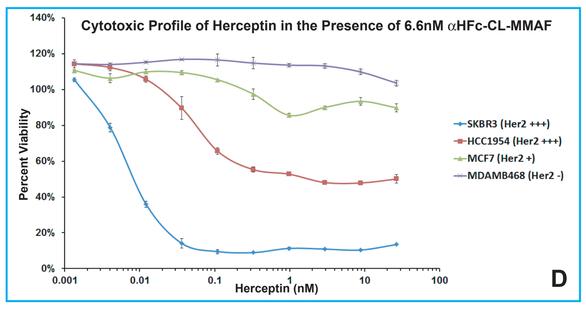


Using Herceptin, which targets Her2-overexpressing tumors cells as an example, we tested cytotoxicity of naked Herceptin in four breast cancer tumor cell lines expressing different amount of Her2 marker in the presence and absence of the 2°ADCs. SKBR3 and HCC1954 are Her2 overexpressing cells, MCF7 has normal Her2 expression, and MDA-MB468 is Her2 negative. *In vitro* SKBR3 is slightly sensitive to unconjugated Herceptin treatment, while HCC1954, MCF7, and MDA-MB468 are resistant to naked Herceptin (**A**). The unconjugated anti-human IgG Fc antibody (αHFc) does not change the apparent effect of Herceptin against these cell lines (**B**). In contrast, in the presence of constant amounts of anti-human IgG Fc MMAF conjugates with noncleavable linker αHFc-NC-MMAF or cleavable linker αHFc-CL-MMAF, Herceptin displays potent cytotoxicity against both Her2-overexpressing SKBR3 and HCC1954 cells, while showing little killing of Her2 normal MCF7 or Her2 negative MDA-MB468 cells (**C** & **D**). Similar to the MMAF conjugates, the other 2°ADCs in combination with naked Herceptin also show specific cytotoxic profiles against the Her2 over-expressing cells, but not the Her2 normal or negative cells.

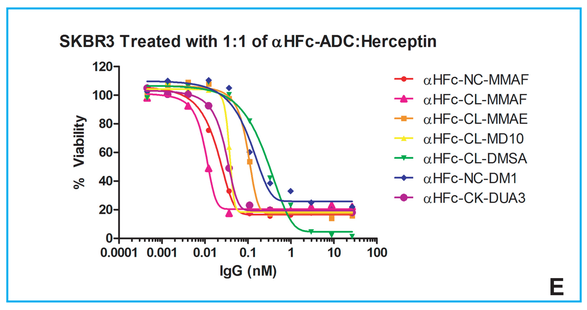


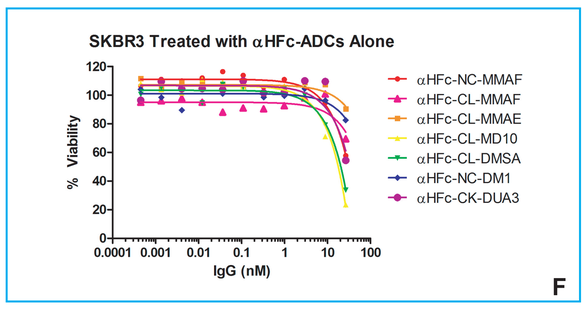


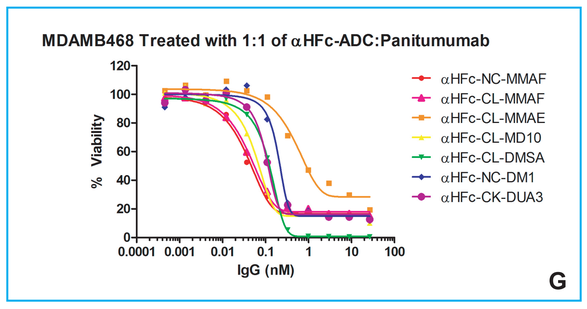


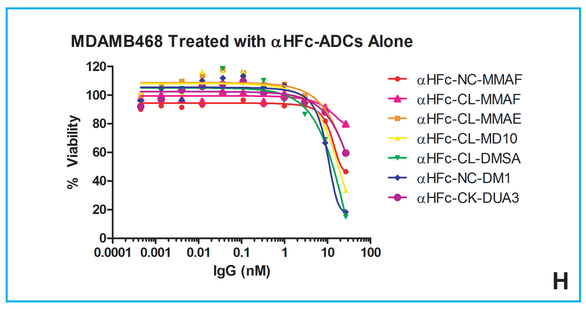


The anti-human Fc ADCs (αHFc-ADCs) are ideal for pre-screening antibodies with a human IgG Fc moiety or recombinant proteins with a human IgG Fc fusion to determine their cytotoxicity. Moradec offers a variety of αHFc-ADCs with cleavable (CL) or non-cleavable (NC) linker to different cytotoxic drugs, such as MMAE, MMAF, MD10, DM-1, DUA3, and DMSA, etc. These 2°ADCs are specific and effective screening tools. For instance, the αHFc-ADCs display specific and potent killing of the Her2 overexpressing SKBR3 in combination with naked Herceptin (**E**), while show little killing in the absence of Herceptin (**F**). Similarly, the αHFc-ADCs display specific and potent cytotoxicity against the EGFR overexpressing MDAMB468 in the presence of EGFR-specific Panitumumab (**G**), while show little killing alone (**H**). When applied in combination with tumor specific monoclonal antibodies or recombinant Fc fusion proteins, the αHFc-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 conjugates.

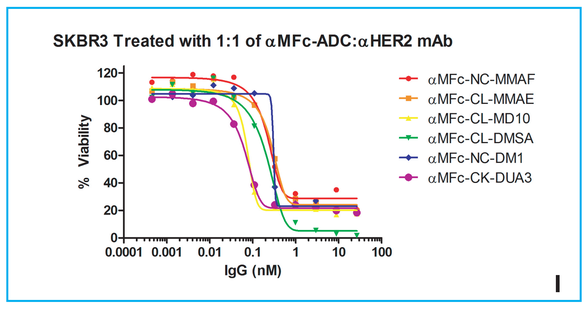


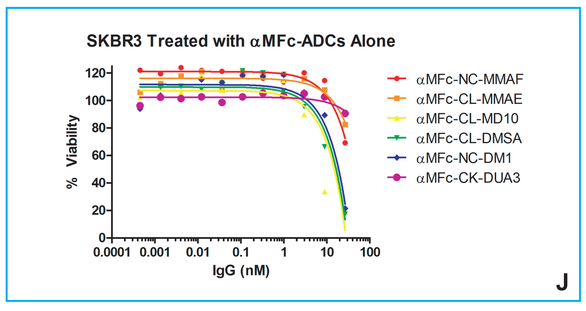






The anti-mouse Fc ADCs (αMFc-ADCs) are ideal for pre-screening antibodies with a murine IgG Fc moiety or recombinant murine IgG Fc fusion proteins to determine their cytotoxicity. For example, potent killing can be achieved with αMFc-ADCs against the Her2 overexpressing SKBR3 in combination with an unconjugated mouse anti-Her2 mAb (**I**), while show little killing in the absence of the primary antibody (**J**). Hence, screening your hybridoma directly has become much easier using Moradec's αMFc-ADCs.





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. Not only can the 2°ADCs be used for screening antibodies, they can also be applied to recombinant protein ligands for targeting cell-surface receptors. This is particularly useful in the early proof-of-concept study for targeting cell-surface receptors in the absence of known monoclonal antibodies.

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, since the 2°ADCs are conjugated with well sought-after cytotoxic drugs such as potent tubulin inhibitors or DNA damaging molecules, testing relevant MOA 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.