White Paper

Development of First-in-Class Novel Immuno-Oncology Therapeutics Targeting Mucin 1-C (MUC1-C)

MUC1-C: Unique target for many tumors

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Executive Summary

- **Oncology has been rapidly evolving in recent years**. We are moving to a much more targeted approach than chemotherapy, via the use of monoclonal antibodies (MAb) which bind with antigens that are expressed specifically on target tumor cells without hurting healthy cells.

- **MUC1 is overexpressed in most types of cancer & therefore is a very attractive high- profile target for immuno-oncology:** Our target is MUC1, an oncoprotein highly (over)expressed in more than 80% of solid tumors. Around 1 million patients with solid tumors express aberrant MUC1 annually in USA. These tumors include metastatic breast cancer, triple negative breast cancer, non-small cell lung cancer, small cell lung cancer, ovarian, liver, gastric, pancreatic, neuro-endocrine prostrate and other cancers.

- **Immune Privilege Site – leading to low toxicity**: In contrast to healthy cells, which only express MUC1 at their apical border, tumor cells lose their apical-basal polarity and overexpress MUC1-C over their entire cell surface. The apical expression of MUC1 shields healthy cells from being targeted by MUC1-C-specific targeting drugs.

- **Novel, first-in-class anti-MUC1-C antibodies.** We are the front runners in this field and have a platform of 1st- and 2nd-generation products undergoing preclinical as well as clinical studies.

- **Independent academic and corporate validation.** The National Cancer Institute (NCI) through their NExT program, has tested and is advancing the IND enabling studies, including scaleup and GMP production with our MUC1-C ADC for US-FDA submission.

- **Phase 1 trials.** POSEIDA Therapeutics Inc has licensed our MAb for use with their chimeric antigen receptor T cells (CAR-T) program. Poseida has already started Phase 1 trials with some early results demonstrating excellent human safety. In Aug 2023 Astellas paid 50 million usd for First look rights for the MUC1-CAR-T program to Poseida.

- **Selectivity and Specificity of MUC1-C MAbs.** We have optimized our final MAb candidates using both knock-in and knock-out models, and we have confirmed biological activity in syngeneic, xenograft, transgenic and patient-derived xenograft (PDX) models.

- **We have a robust Investigative Product (IP) portfolio.** Intellectual property portfolio consisting of different therapeutic approaches for multiple indications based on the unique MUC1-C monoclonal antibodies.

- **Excellent Team-** High profile, well regarded experienced team as well as expert advisors are on board.

The transformational role of immunotherapy in oncology

It has been over 100 years since Paul Ehrlich first proposed the concept of a “magic bullet” when describing the properties of antibodies[20]. However, it was not until 1975 that the therapeutic potential antibodies began being unlocked, after the development of the hybridoma technique in 1975, which allowed scientists and clinicians to develop and isolate large amounts of specific clonal antibodies on demand[21]. Since then, monoclonal antibodies slowly found their way into the arsenal of clinicians working in targeted cancer immunotherapy. To date, several antibodies have been developed and tested and are now routinely used in the clinic.

The antibodies can be designed to specifically bind an antigen present on target tumor cells while sparing healthy tissue and then attack them through multiple mechanisms. One of the mechanisms is by using a cytotoxic drug (toxin) to induce cell death of target cells. Antibodies are conjugated with cytotoxic drugs (toxin) to form ADC (Antibody drug conjugate). Upon binding to the antigen expressed on the surface of tumor cells, ADCs are internalized and release the cytotoxic drugs directly into the cytosol, leading to cell death **(Figure 1).** This strategy allows for the preferential killing of tumor cells while sparing the healthy tissue of the patient of otherwise nefarious side effects.

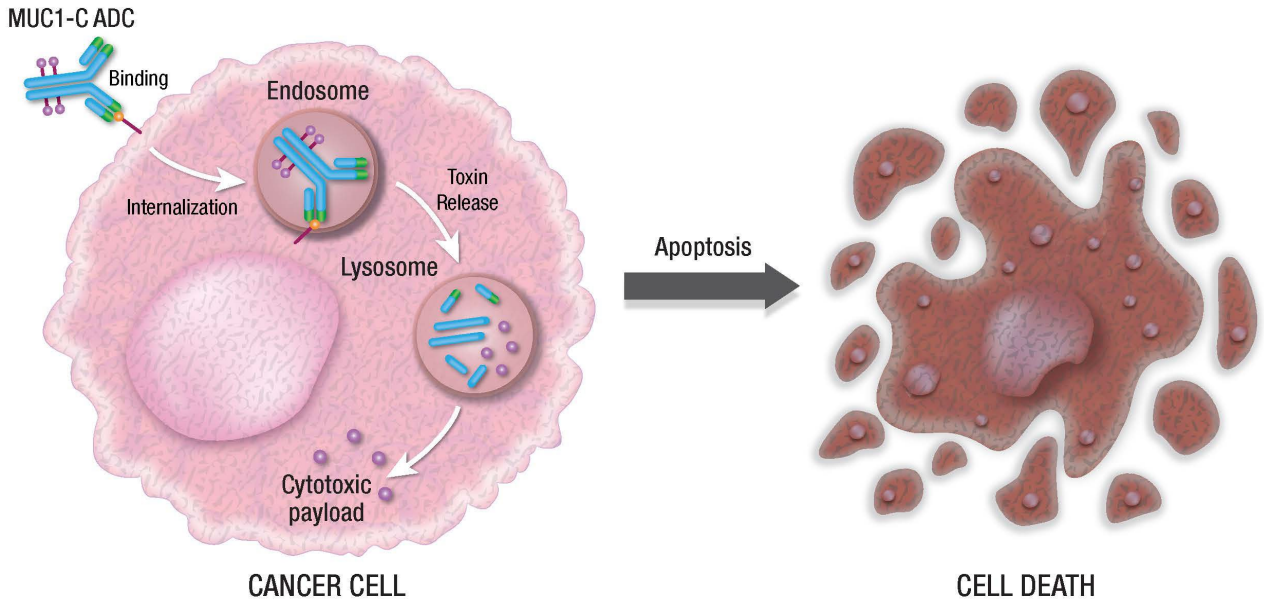


Figure 1: Schematic depiction of an antibody carrying a cytotoxic drug being internalized by a cell expressing the target antigen. Upon internalization, the toxic payload is delivered to the cytosol, leading to apoptosis.

The Achilles' heel of antibodies: how to combine specificity with tumor heterogeneity? The XYone breakthrough

The heterogeneity of tumors must be taken into consideration when it comes to the rational design of an antibody to be used in cancer immunotherapy: different types of cancer are characterized by (over)expression of different antigens. As an example, while an ADC targeting HER2 can show remarkable efficacy against breast cancer, it is unlikely to improve the outcomes in patients with neuro endocrine prostate cancer (NEPC), because NEPC cells do not express the HER2 protein. Therefore, one of the key goals of cancer immunotherapy is the development of an antibody that recognizes an antigen with widespread expression across different types of cancer cells but not in healthy cells and tissues. Cancer cells often metastasize across organs while retaining their distinct character. Widely expressed antigen would be a superior target than a narrowly expressed antigen. XYone' s target oncoprotein is MUC1. The expression of MUC1 has been validated in over 80% of all solid tumors (Table 1), paving the way for the development of our first-in-class panel of antibodies against the extracellular domain of MUC1. Over a million patients are diagnosed annually with tumors over-expressing MUC1 in USA . Our platform of drugs could potentially help them all.

Cancer Type	USA Incidence	Deaths	MUC1 +
Breast	284200	44130	91%
Colorectal	158590	54410	81%
Esophageal	19260	15530	69%
Gastric	26560	11180	77%
Ovarian	21410	13770	83%
Prostate	248530	34130	79%
Pancreatic	60430	48220	81%
NSCLC	235000	131880	99%
Bladder	83730	17200	83%
Kidney	76080	13780	84%

Table 1: Estimates of Cancer Types, annual deaths and MUC1 over-expression.

Approval of XYone's first drug for any one cancer indication will have the potential to become the most ubiquitous cancer drug either as monotherapy or combination therapy.

MUC1 – A unique ubiquitous specific antigen on Tumor Cells

MUC1 is a protein expressed on the apical surface of normal epithelial cells[4] (Figure 2), facing the external environment in the respiratory and gastrointestinal tracts and lining ducts of specialized organs including the breast, liver, prostrate, pancreas, lungs, and kidneys etc. MUC1 protects the body from infection by binding to a wide range of pathogens and by regulating inflammatory response to infection.

Upon malignant transformation of the epithelial cells, MUC1 gets significantly over expressed (5X-100X) by the tumorigenic cells. Furthermore, epithelial cancer cells lose their apical-basal polarity, and overexpress MUC1 over the entire cell surface[16,17], where MUC1 starts functioning as an oncogene[1-3]. MUC1 interacts with other oncogenes and their respective signaling pathways, substantially promoting cell growth and inhibiting cell death in cancer cells[5–9]. MUC1 has been implicated in many hallmarks of cancer, such as cell invasion, migration, adhesion, proliferation, and resistance to apoptosis and chemoradiotherapy.[10]

Many other companies and researchers have attempted to target MUC1 as an anti-tumor immunotherapeutic strategy in patients with MUC1+ cancers, but clinical trials were largely ineffective and

unable to show a clear improvement in outcomes[11,12]. To understand what makes XYone's strategy unique, one must understand the structure of MUC1. MUC1 consists of two subunits, MUC1-N and MUC1-C [(1-3)]. MUC1-N, is a highly glycosylated mucin component shed from the cell surface into circulation upon binding while the MUC1-C component remains anchored to the cell surface (Figure 2**)**. It is hypothesized that earlier attempts at targeting MUC1 failed to show clear clinical benefits because the antibodies used targeted the MUC1-N domain, which is shed from the surface of cancer cells into circulation[12]. The MUC1-N in sink can be significant amount. If the antibodies were to bind to the shed MUC1-N, the payload toxin would not reach the targeted cancer cells and thus the drug had poor efficacy [12,17–19]. By targeting the MUC1-C domain, XYone Therapeutics bypasses this pitfall and attacks the part that cannot be shed and delivers the payload directly into the tumor cells.

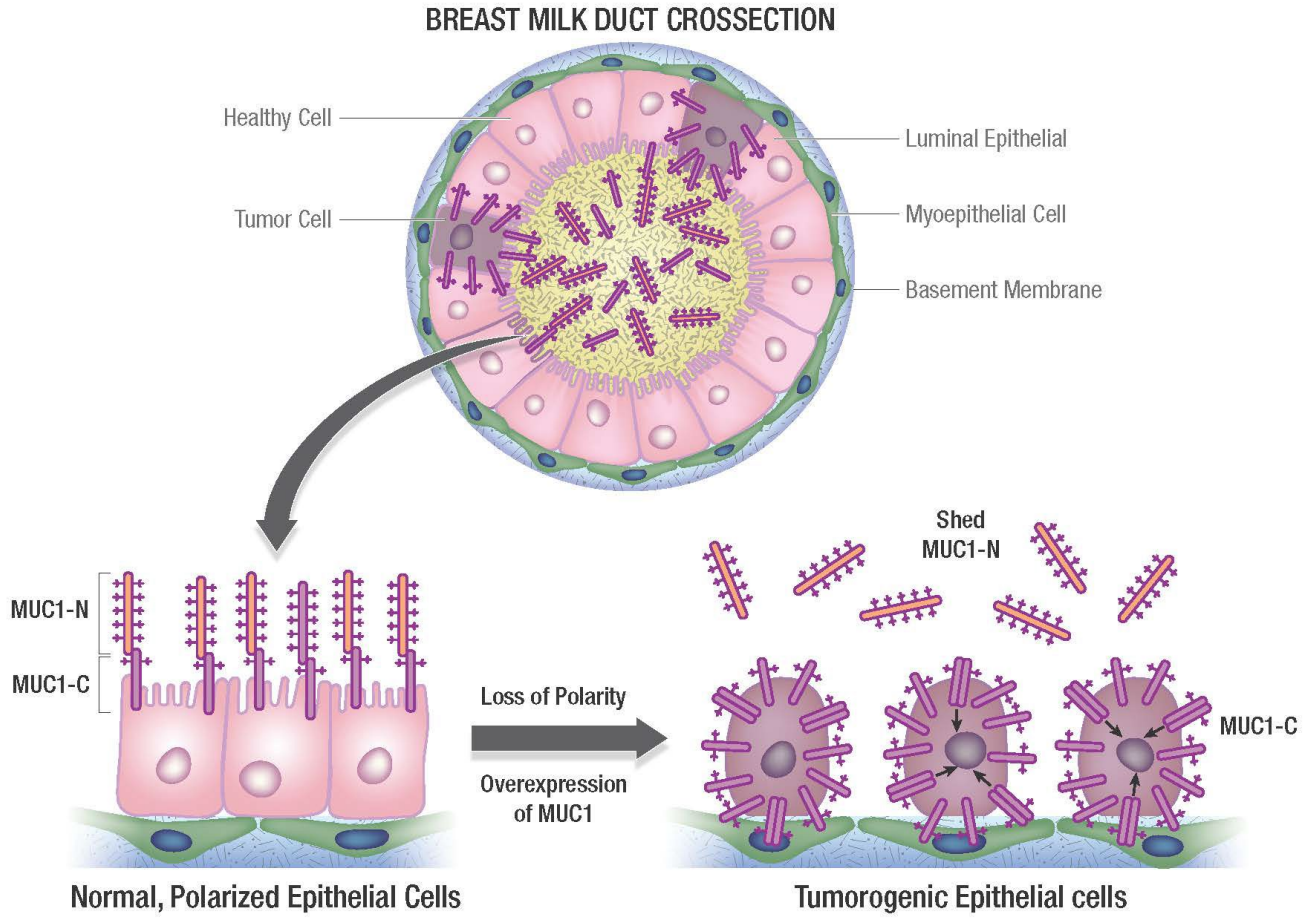


Figure 2: In epithelial cancer, epithelial cells lose their apical-basal polarity, shed MUC1-N, and overexpress MUC1-C over their entire cell surface[16,17].

MUC1-C interacts with other oncogenes and participates in their downstream signaling pathways to substantially induce tumor cell growth and block cell death of cancer cells [5-7]. It is the Achilles heel of the elusive MUC1 target -which was identified as one of the best potential targets out of 75 tumor associated antigens way back in 2014 by NCI in one of their studies.

MUC1 drives multiple hallmarks of the cancer cell including i) increased growth, ii) inhibition of cell death, iii) metabolic alterations, iv) epigenetic reprogramming, v) chromatin remodeling, vi) evasion of cancer stem cell (CSC) markers, vii) the CSC state, self-renewal capacity and tumorigenicity, viii) drug resistance and ix) immune evasion.

The aberrant overexpression of MUC1-C in diverse human malignancies, the interaction between the MUC1-C subunit and multiple effectors associated with transformation into cancer cells, and the demonstration that the MUC1-C is sufficient to induce the transformation support the notion that MUC1-C is a highly attractive target for cancer treatment[8]. The findings that MUC1-C induces gene signatures that are predictive of poor outcomes for patients with breast and lung cancer have provided further support for the importance of MUC1-C as a unique therapeutic target[9].

5. MUC1-C represents a unique opportunity for the selective killing of cancer stem cells.

Cancer stem cells (CSCs) are a minority population of cells within a tumor that - much like a normal stem cell - have the potential for self-renewal and differentiation, giving rise to many of the other cell types that constitute the tumor.[20] Today, CSCs are believed to be one of the key players that drive the resistance of tumors to therapy, contributing to relapse. As such, despite being a minority population within a tumor, CSCs must be effectively targeted by curative tumor immunotherapies.

The presence of tumor-infiltrating lymphocytes (TILs) in the tumor microenvironment (TME) is associated with improved patient survival in response to chemotherapy.[21] One of the ways through which CSCs protect themselves from immune clearance by TILs is through the up-regulation of PD-L1.[22,23] Blocking PD-L1 expression by antibodies is the fundamental basis for the recent success of therapeutics termed immune checkpoint inhibitors (ICIs), such as pembrolizumab, which aim to ablate the negative signaling downstream of PD-1(partner protein to PDL-1) in immune cells as a way to retain their tumor-killing activity[25]. The responsiveness of cancer cells to (ICIs) is also improved by increases in TIL density indicating that immune cell-depleted “cold” TMEs associate with resistance to treatment with chemo or ICIs.

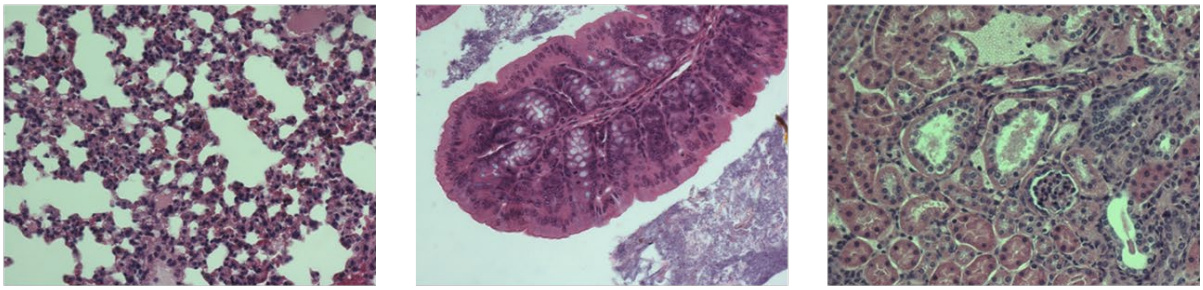
MUC1-C activates the PD-L1 gene in human cancer cells (13). Targeting MUC1-C suppresses PD-L1 expression and is associated with increases in TILs in the TMEs and tumor cell killing. Thus MUC1-C targeting helps overcome the growing resistance to ICIs in relapsed tumors as well as potentially increase their efficacy as a combination drug with the ICIs. The MUC1-C functions as an oncoprotein in cancer stem cell (CSC) progression and is a potential druggable target for solid tumor treatment[9].

6. MUC1-specific anti-cancer immune responses DO NOT cause autoimmune damage to normal MUC1-expressing tissues – The “Immune Privileged” attack.

Mucins, such as MUC1, are constitutively expressed at the cell surface of nearly all epithelial cells protecting the apical border in respiratory and GI tracts as well as in the ducts of specialized organs such as breast, liver, prostate etc. Their canonical function is to protect cells from infection, by working as a “decoy barrier” that can be released from the cell membrane into the lumen to promote the excretion of pathogens. This outside-facing location of MUC1 naturally makes healthy cells immune to drugs administered through systemic circulation, such as antibodies. By contrast, induction of humoral and cytotoxic T-cell immunity against MUC1 is highly effective against cancer cells that have lost apical-basal polarity and express MUC1 across the entire cell surface.[28,29] These findings strongly demonstrate that MUC1 is an immune privileged site at the apical borders of normal epithelia [16,17]. ADC- and CAR-T cell-based therapies against MUC1-expressing tumors should have highly favorable toxicity profiles towards normal tissues and healthy cells while targeting tumor cells efficaciously.

As further evidence of the immune privileged site at the apical border, we analyzed the organ tissue of MUC1 Transgenic mice treated with one of our ADC’s at a high dose. There was no evidence of overt toxicity in any of the organs tested (Figure 3). These findings indicated that the healthy epithelial cells did not bind to the ADC and the healthy tissue was not adversely affected despite a supratherapeutic dose.

Analysis of tissues from MUC1.Tg mice treated with hMAb 3D1-MMAE ADCs at high dose



LUNG COLON KIDNEY

Figure 3: hMAb 3D1-MMAE ADCs were injected i.v. to MUC1.Tg mice at a dose of 15 mg/kg. On day 7, lung, trachea, heart, thymus, thyroid, stomach, small intestine, colon, liver, pancreas, spleen, ovary, prostate, brain, spinal cord and leg muscle were fixed in Bouin's solution. Tissue sections were stained with H&E and examined by microscopy. There was no observed toxicity to these tissues. Representative H&E sections of the lung, colon and kidney are shown

7. **Independent Commercial and Academic validation of XYone' s lead asset (human monoclonal antibody MAb-h3D1)**.

The generation and characterization of the first-in-class novel anti-MUC1-C antibody [h3D1] provided an opportunity for the development of therapeutic agents targeting this oncogene [18.] Anti-MUC1-C CAR-T cell approach using h3D1 sequences was highly effective against triple negative breast cancer (TNBC) and ovarian cancer xenografts (Figure 4) and has entered the clinic (currently Phase 1 trials) through a licensing deal with POSEIDA Therapeutics Inc for the treatment of TNBCs and other MUC1-C expressing cancers (NCT05239143: P-MUC1C-ALLO1 Allogeneic CAR-T Cells in the Treatment of Subjects with Advanced or Metastatic Solid Tumors).

We have developed our first ADC by conjugating MAb XY with a cleavable linker to a toxin(undisclosed) 01. The linker and toxin have been widely used in approved ADCs, such as Adcetris (Seattle Genetics), Polivy (Roche Genentech) and Padcev (Astellas), as well as in other ADCs under development.

Our studies have confirmed that XYA01 ADCs were highly potent in killing MUC1-expressing cancer cells in vitro, with an IC_{50} (the inhibitory concentrations that inhibited 50% of cells) in the sub-nanomolar (nM) range[18]. The XYA01 ADCs were also effective against established human tumor xenografts, including TNBC PDX models, without evidence of overt toxicity[18].

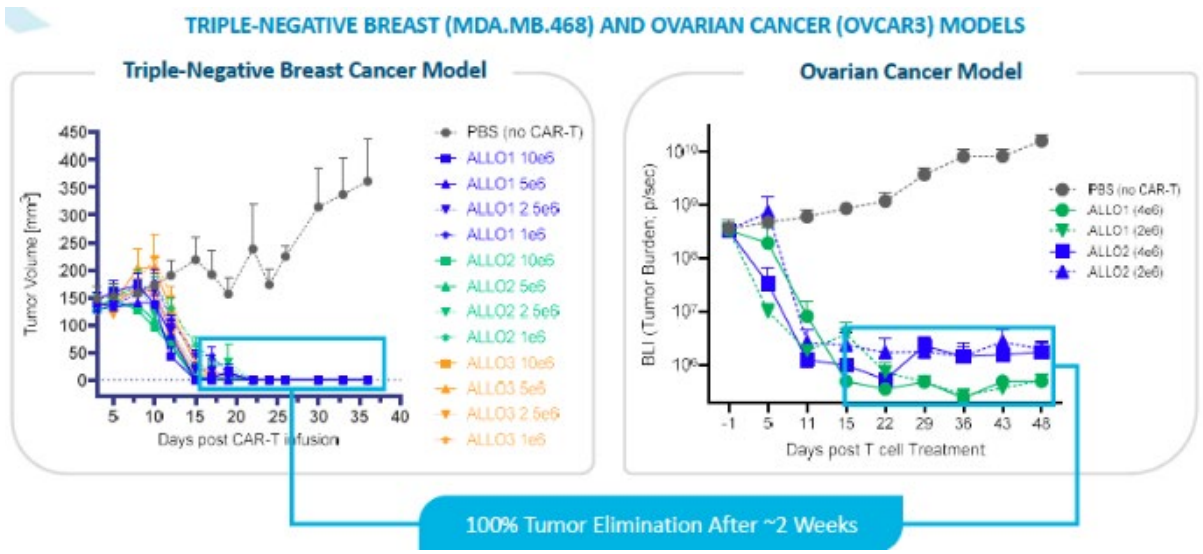


Figure 4: An anti-MUC1-C CAR-T cell approach using h3D1 sequences was highly effective against triple negative breast cancer (TNBC) and ovarian cancer xenografts.
Source Poseida Presentation

To confirm the absence of toxicity, studies were also performed in humanized MUC1.Tg mice that express human MUC1 at a level and pattern found in humans[18]. Studies in the MUC1.Tg mice model demonstrated no significant adverse effects of the XYA01 ADC on different organ tissues or hematologic and blood chemistry parameters. These findings supported the notion that the MUC1-C-specific epitope is selectively exposed on the surface of carcinoma cells as compared to normal epithelia.

Based on this therapeutic selectivity, XYA01 ADC is now under development by the **NCI** under **NExT Program,** which is conducting IND-enabling studies and will conduct early phase trials in patients with TNBCs. An example of XYA01 xenograft studies in ER+ breast cancer conducted by XYone is shown in Figure 5.

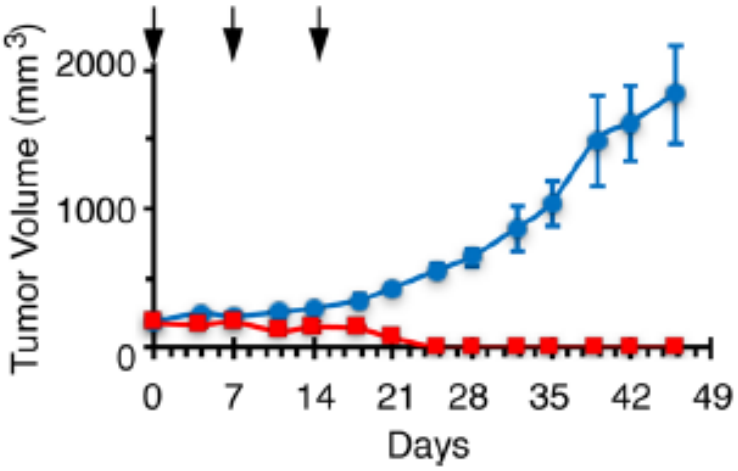


Figure 5: Tumor volume across a 49-day experiment: administration of XYA01 ADC was associated with a full reduction in tumor volume (red points), while the control group experienced significant tumor growth over the same time period (blue points). The arrows indicate the timing of the three doses.

8. MUC1-C anti-cancer onslaught using unique PLATFORM

The ADC drugs rely on different payloads for sensitivity to different cancer indication. Our novel ADCs are under evaluation for targeting MUC1-C along with cancer indication-specific payloads. We are advancing two disease specific cytotoxic payloads to make two ADCs XYA01 XYA02. These are extremely potent cytotoxic drugs characterized by an IC_{50} (the inhibitory concentrations that inhibited 50% of cells) in the sub-nanomolar range, and a very unfavorable toxicity profile if administered free drug systemically.

We are currently employing XYA01 to treat ER^+ breast cancer and the XYA02 to treat relapsed/refractory (R/R) metastatic colorectal cancer (R/R-mCRC)

Interestingly, XYA02 is ~10-fold more potent in R/R-mCRC **(Figure 6, left)** and ~12-fold more potent in TNBC **(Figure 6, middle)**, as compared to XYA01. Instead, XYA01 appears to be 3-fold more potent than XYA02 in killing ER^+ breast cancer cells TNBC **(Figure 6, right)**.

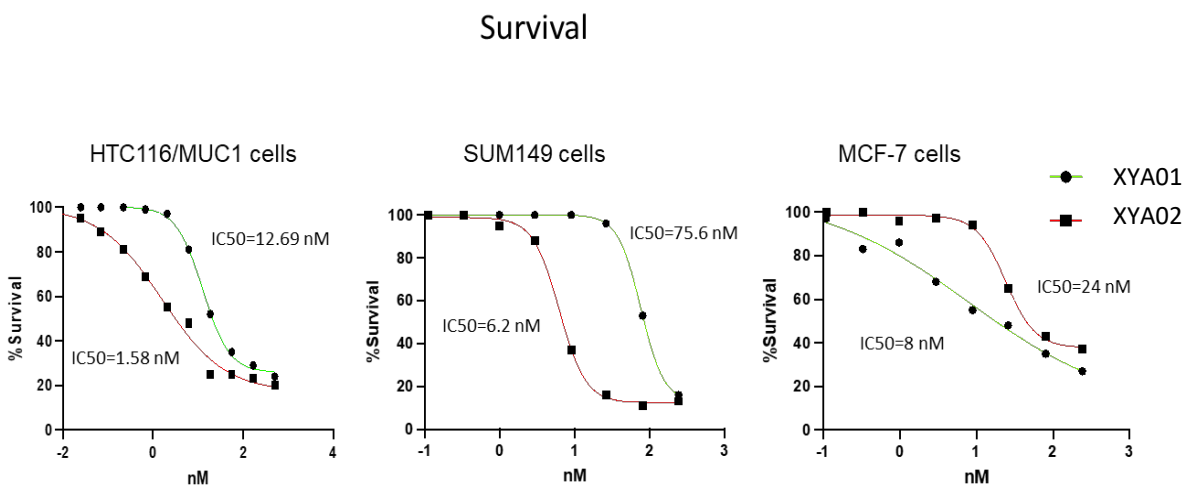
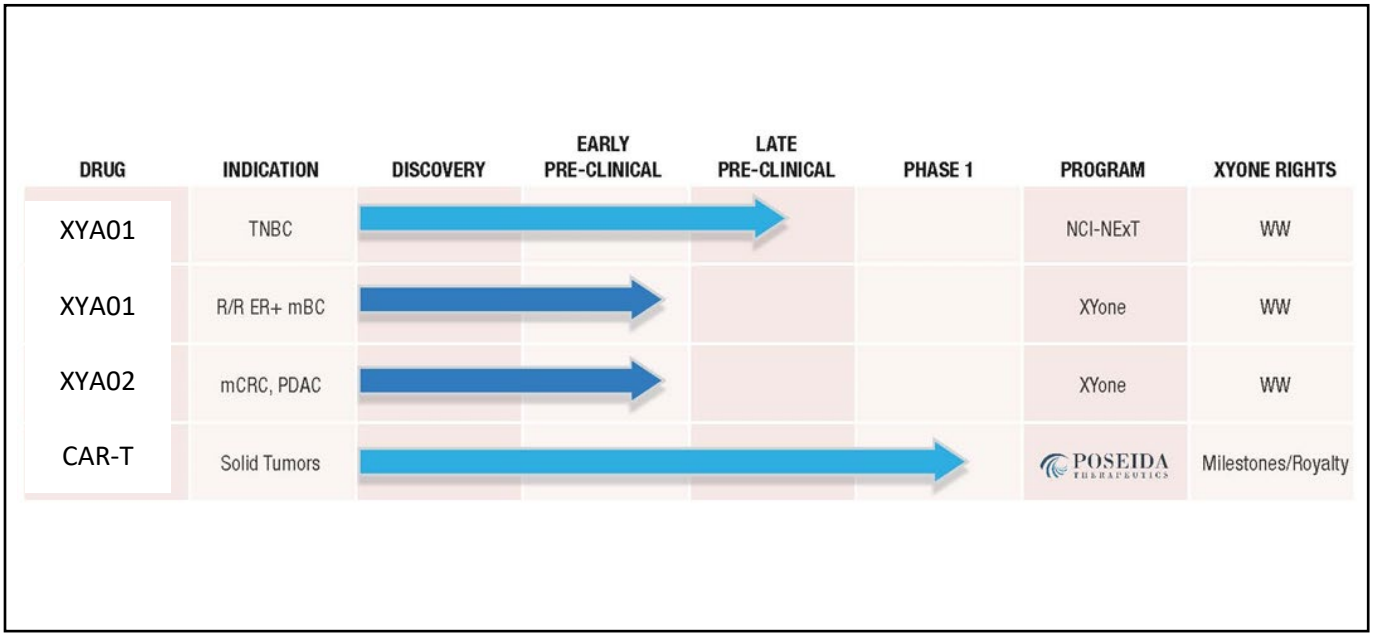


Figure 6: HCT116/MUC1 cells (left), SUM149 cells (middle) and MCF-7 cells (right) were incubated with the indicated concentrations of XYA01 ADCs (green circles) or XYA02 (red squares). The IC_{50} values are indicated in the respective figures.

Additionally, we are also investigating other antibodies that we have discovered as well as the second-generation products using these antibodies such as bi-specifics and bi-paratopic through preclinical work. Further details are available from the company.

10. Pipeline.



11. Robust Intellectual Property Portfolio. XYone has filed multiple patents globally and have extensive coverage world-wide. Twenty five issued and seven pending US and international patents for the MUC1 target, peptide composition of matter, antibodies, and methods of use. Antibodies against MUC1-C patents filed on 2014, 2020 and 2021 for US patents.

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