

Novel Approaches Designed to Enhance the Efficacy of Cancer Treatments by Suppressing Mechanisms of Tumor Metastasis and Chemoresistance

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Inhibiting GAS6/AXL signaling pathway offers a novel approach to addressing cancer's metastatic and invasive properties.

- AVB-500 has been engineered to have higher affinity for GAS6, and is designed to inhibit the AXL protein cascade to halt signaling involved in activation, migration and invasion of cancerous cells.
- AVB-500's benign toxicity profile could be ideal for lowering a patient's treatment burden while maintaining a higher quality of life.
- Compelling early efficacy signal was observed in Phase 1b study of AVB-500 platinum-resistant ovarian cancer.
- AVB-500 has potential to treat multiple different aggressive forms of cancer, including ovarian and clear cell renal cell carcinomas.

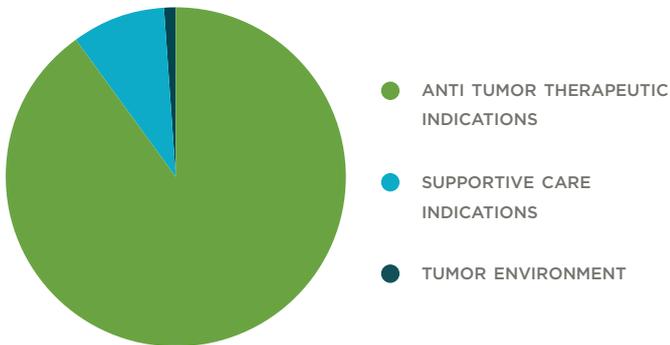
Cancer is one of the most difficult and deadliest diseases to treat, largely due to the complex genetic and molecular framework it involves. Mutations in a number of genes and molecular pathways that invoke immune evasion, cellular metastasis, migration and invasion, allow cancerous cells to recur in the body after an individual has already undergone treatment. The ability of cancerous cells to develop resistance mutations and circumvent treatment requires that treatment be approached from multiple angles, taking the many factors that contribute to disease progression into account.

While the oncology field has seen tremendous advancements in recent decades, there are certain cancers that have not seen an appreciable increase in overall survival of patients. This includes ovarian cancer, one of cancer's most aggressive and fast-acting forms, in which drug development has largely focused on anti-proliferative approaches to treating the disease (American Cancer Society). Yet, research has shown that it is not rapidly dividing cells that leads

to the majority of patient deaths, but rather, it is the metastasis and invasion of cancerous cells into the bloodstream and healthy tissue. Despite this, there are currently no truly effective chemo-sensitizing or anti-metastatic drugs available on the market today.

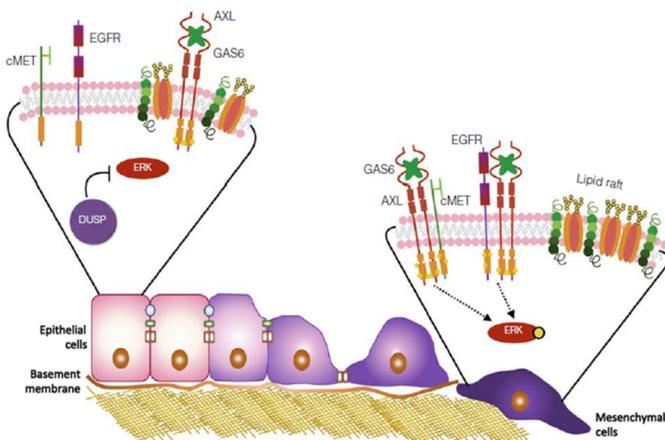
To combat cancer and its multi-faceted nature, treatments that extend beyond proliferation are needed, specifically, approaches that address metastasis, recurrence and treatment resistance. Given the toxicity of our current anti-cancer arsenal, and that many cancer treatments work most optimally when used in combination with one another—leveraging the ability to attack the cancer in a multitude of ways—it is important that new approaches have little-to-no associated toxicity and don't interact with other anticancer agents. This would allow them to be either combined with existing, more toxic treatment modalities, or used for long-term maintenance without negatively impacting a patient's quality of life in a significant way (Aguilera et al. 2016).

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GAS6/AXL: The Path to Metastasis

Recent developments suggest targeting the GAS6/AXL signaling pathway offers a novel anti-metastatic, chemo-sensitizing approach with limited to no associated toxicity (Paccez et al. 2015) (Liu et al. 2010) (Ou et al. 2011) (Ghosh et al. 2011) (Van Ginkel et al. 2004) (Vajkoczy et al. 2006). The activation of the GAS6/AXL pathway leads to a number of cancerous cell hallmarks, including metastasis, acquired drug resistance, fibrotic response and evasion by the immune system (Graham et al. 2014). GAS6/AXL signaling has been shown to be largely upregulated in cancerous cells, notably those that are particularly aggressive and invasive (Koorstra et al. 2009) (Rankin et al. 2014).



In binding to the AXL proteins embedded into the cellular membrane, GAS6 signaling drives the epithelial-to-mesenchymal cell transition and enables cells to survive under less than ideal circumstances, (eg, hypoxia, low pH, low nutrients) (Rankin et al. 2014), a cellular survival program that cancer cells harness in response to a hostile tumor microenvironment.

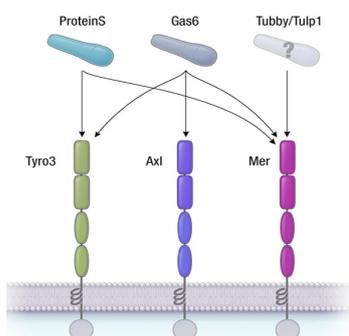
As cancer is a multi-faceted disease controlled by a number of different genetic and molecular factors, inhibiting the GAS6/AXL pathway may provide a promising anti-metastatic and chemo-sensitizing approach that is complementary to established cancer treatments. Certain preclinical research has demonstrated that GAS6/AXL pathway inhibition may increase the efficacy of existing therapeutic regimens (Hugo et al. 2016) (Aguilera et al. 2016). This is scientifically validated by the critical role that AXL plays in metastasis for a broad spectrum of cancers, including ovarian.

Those with advanced metastatic disease express significantly higher levels of AXL, as do those which have developed treatment resistance to prior therapies—including both targeted and cytotoxic treatments. It is important to note that GAS6 is also expressed at higher levels in this patient population.

While many cancer therapies can be incredibly toxic to patients with a host of side effects, inhibiting the GAS6/AXL pathway may be innocuous by comparison based on GAS6 knock out mouse experiments that demonstrated no abnormal phenotype.¹

The TAM Family of Tyrosine Kinases

The AXL molecule belongs to the TAM family of receptor tyrosine kinases (RTKs). There are three members of the TAM family, Tyro3, AXL and Mer (Liu et al. 1998) (O'Bryan et al. 1991). These proteins are responsible for driving a number of key cellular processes, including cellular proliferation, vascularization and immune cell recognition (Linger et al. 2008). The overactivation of TAMs has obvious implications for the proliferation of cancer.



TAMs have a unique structure compared to previously discovered receptor tyrosine kinases (Nagata et al. 1996). There is a transmembrane domain (TM) that crosses the plasma membrane on the cell. The tyrosine kinase domain is found on the inside of the cell, and ligand binding occurs on the outside of the cell. In the absence of the ligand, the TAM molecule exists as a monomer. Upon binding of the ligand, TAMs dimerize, and the pair of receptors becomes activated. The active form of the molecule can then phosphorylate a whole host of downstream proteins, setting off a very large signal transduction cascade in cells.

The TAMs are activated by ligands. The Mer and Tyro3 kinases are activated by both GAS6 and Pros1, which are approximately 50% identical. (Liu et al. 1988). Pros1 is abundant in most cells and in the blood, as it plays an important anticoagulant role in the blood coagulation cascade (Linger et al. 2008). AXL can only be activated by GAS6, making the GAS6 ligand an attractive target for therapeutic intervention (Nagata et al. 1996).

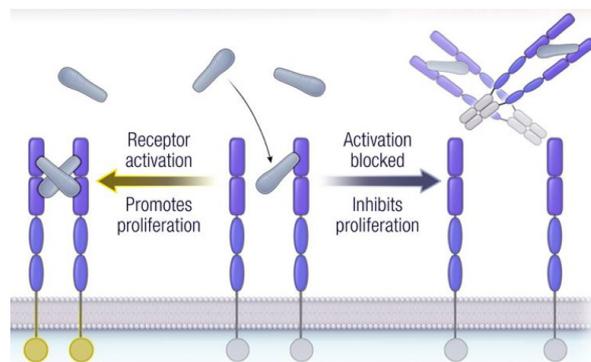
Overcoming Challenges to Targeting GAS6/AXL Pathway

Despite the promise that inhibiting the GAS6/AXL pathway holds in targeting the overlooked aspects that often contribute to cancer progression, there are a number of factors that have made it difficult for drug developers to target and control. First, there is an incredibly high affinity between the GAS6 ligand and the AXL receptor protein (>30 pM), making it challenging to target and inactivate the AXL pathway directly (Nagata et al. 1996).

This mechanistic barrier has led to a number of failures and suboptimal results when homing in on the GAS6/AXL pathway. For instance, antibody approaches are not capable of binding either AXL or GAS6 tightly enough, rendering them unable to outcompete the ligand binding to its target. Small molecule approaches also do not have high enough affinity and also lack selectivity and specificity to the AXL proteins themselves, as there is a significant homology and conservation amongst the binding pocket of receptor kinases. This often leads to off-target effects, associated toxicity and a greater potential for treatment resistance to develop (Graham et al. 2014).

In order to successfully target the GAS6/AXL pathway without off-target and potentially toxic effects, it is crucial to focus on the GAS6 protein itself, given that this is the only ligand capable of binding to and activating the AXL receptor protein. AVB-500, does exactly that. A novel, soluble Fc-fusion protein, this ultra-high affinity decoy protein captures both the bound and circulating GAS6 ligand, ultimately shutting off AXL signaling within the cell.

AVB-500 is essentially a decoy protein made from the GAS6 binding portion of the AXL protein that has been mutated to further increase its already high affinity for GAS6. The affinity of AVB-500 for GAS6 is 150 femptomolar: 200-fold greater than that of natural GAS6/AXL affinity. Ultimately, in binding to GAS6 and inhibiting the AXL protein cascade, AVB-500 has the potential to address metastatic processes by mitigating the activation, migration and invasion of cancerous cells.



AVB-500: A Proven, Nontoxic Disruption to GAS6/AXL Signaling

AVB-500 has demonstrated a number of antitumor effects in preclinical studies. When used in combination with pegylated liposomal doxorubicin (PLD), AVB-500 prevented tumor cell growth and metastasis, even in carboplatin-resistant cells. AVB-500 exhibited similar tumor growth inhibition when used in combination with Taxol, even in platinum-resistant cells. These results were replicated in patient-derived tumor xenograft models. When AVB-500 was used as a maintenance treatment following discontinuation of chemotherapy, the inhibition of tumor growth persisted.

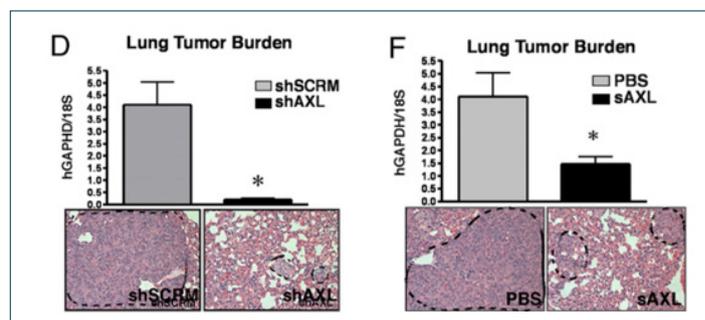
In addition to the preclinical data supporting AVB-500's ability to inhibit cancer progression, AVB-500 has an exceptional safety and tolerability profile, supporting its potential use in combination with existing treatments or as a long-term maintenance therapy. In preclinical models, AVB-500 was administered at 30-times the pharmacologically-active dose, without causing toxicity. In fact, the safety profile for AVB-500 was favorable enough to allow an initial clinical study in healthy volunteers. AVB-500 follows the kinetics of target-mediated drug disposition.

The culmination of these data indicate that AVB-500 dosing could be conveniently adjusted to match the dosing frequency of other chemotherapies or targeted therapies being used in combination with one another. Most importantly, this low toxicity could be ideal for lowering a patient's treatment burden while maintaining a higher quality of life.

Targeting the Multi-Faceted Nature of Cancer

We are at an inflection point in developing cancer treatments, in which the disease must be treated by addressing the multitude of complex factors that contribute to its progression. Within the oncology sector, there exists a significant need for anti-metastatic and chemo-sensitizing therapies, which would bolster the industry's existing drug arsenal and potentially provide more effective and targeted therapies. Development of the next-generation

of therapies need to take a patient's quality of life into consideration, ensuring not to contribute to the treatment burden that already exists for those who face poor cancer prognoses.



In patients who face less-than-optimal outcomes due to metastasis and treatment resistance, AVB-500 holds the potential to treat multiple different aggressive forms of cancer, including ovarian and clear cell renal cell carcinomas (ccRCC). Targeting and inhibiting the GAS6/AXL signaling pathway offers a novel approach to addressing cancer's metastatic and invasive properties. Based upon AVB-500's favorable safety profile, coupled with its specifically targeted mechanism of action, the decoy protein offers a multitude of unique properties that allow it to be used both in combination with existing therapies, as well as a maintenance drug. Currently, AVB-500 is being developed in indications in which GAS6/AXL signaling has shown to be strongly implicated in driving metastasis and chemoresistance, including ovarian cancer and ccRCC (Zhang et al. 2008) (Graham et al. 2014) (Wang et al. 2016).

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¹(Lu, Q., Gore, M., Zhang, Q., Camenisch, T., Boast, S., Casagrande F. (1999) Tyro-3 family receptors are essential regulators of mammalian spermatogenesis. *Nature* 398, 723-28; Yanagita et al., *J. Clin. Invest.* 110:239-246 (2002). doi:10.1172/JCI200214861).