Development of Localized Cisplatin Chemotherapy: From Benchside to Investigational New Drug Application

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ABSTRACT SUMMARY
Many cancers preferentially spread to the lymphatics before systemic dissemination. Intralymphatically-targeted cisplatin nanoparticles (HylaPlat™) were developed for treating locally advanced head and neck cancers. This treatment methodology significantly reduced the side effects and increased the efficacy of cisplatin-based chemotherapy in both rodents and canines, compared to conventional intravenous chemotherapies. To benefit many cancer patients with the improved localized cisplatin therapy, we are in the process of preparing an Investigational New Drug Application to the FDA.

INTRODUCTION
Many cancers, such as breast cancer, head and neck cancer and lymphoma, preferentially metastasize via the lymphatic system prior to their systemic dissemination. Conventional chemotherapy has limited penetration into the lymphatics due to its anatomy, because drug molecules have to diffuse from the blood capillaries into the surrounding tissues and further enter the lymph vessels to reach the diseased lymph nodes. Therefore, chemotherapy can be of limited benefit in treating lymph node disease. In addition, due to the systemic administration and wide distribution, conventional intravenous chemotherapy causes a variety of organ toxicities, including but not limited to: renal toxicity, cardiotoxicity, and hepatic toxicity. This limits its use in the clinic, especially for the elderly or late-stage patients who cannot tolerate systemic toxicities. Therefore, there is a critical need to develop a localized drug delivery platform that confines the anti-cancer drugs to the site of the disease.

EXPERIMENTAL METHODS
Cisplatin was chemically conjugated to hyaluronan (HA) to form hyaluronan-cisplatin nanoparticles, HylaPlat™. The nanoparticles were purified and concentrated using an highly efficient and integrated KrosFlo Research IIIi Tangential Flow Filtration system equipped with a hollow fiber tangential flow module, a peristaltic pump, a permeate scale, pressure monitors and automatic back pressure valves.

The cisplatin substitution degree, release kinetics, particle size, molecular weight, viscosity, osmolality, sterility, bioburden, particulate content, impurity profiles, and stability of the formulation were determined. Various excipients were screened. All analytical methods utilized were fully validated according to FDA and/or USP guidelines.

The anti-proliferative activity, cellular uptake kinetics and mechanisms of HylaPlat™ were explored using various cancer cells including human head and neck squamous cell cancer cells MDA-1986, and multiple hyaluronan specific receptors, including CD44.

The pharmacokinetics, biodistribution, toxicokinetics and efficacy of subcutaneously or intratumorally injected HylaPlat™ were evaluated in both rodents and canines. Mice bearing cisplatin sensitive xenografts were treated weekly for three consecutive weeks at 3.5 mg/kg. Canines with spontaneously-occurring limb soft tissue sarcomas were given a single 0.85-mg/kg subcutaneous dose peritumorally. Canines with spontaneously-occurring oral squamous cell carcinoma, oral melanoma, or nasal tumors received 1 to 3 doses at 10 to 30 mg/m², peritumorally.

RESULTS AND DISCUSSION
The cisplatin substitution degree was 10-20 w/w%. HylaPlat™ demonstrated anti-proliferative efficacy similar to standard cisplatin in vitro with sustained release of cisplatin (t₁/₂ =10 h)¹. The characteristics of HylaPlat™ meet FDA requirements for injectable formulations.

HylaPlat™ resulted in higher plasma area-under-the-curve (AUC) and lower Cₘₚₙₐₓ in rats. The AUC of cisplatin in the axillary lymph nodes after injection with HA-Pt increased 74% compared to standard cisplatin². Murine xenografts demonstrated improved response to HylaPlat™ treatment (complete response: 67%; partial response: 33%)
compared to standard cisplatin therapy (partial response: 75%; progressive disease: 25%)\textsuperscript{3}.

Figure 1. Tumor growth in mice (treatments received at week 3, 4 and 5).

The HylaPlat\textsuperscript{TM} increased the canine plasma AUC of cisplatin 5.4 fold and the lymph node concentration 18 fold compared to i.v. cisplatin. The tumor:plasma ratio in canines was 429, indicating the nanoparticles concentrated drug within the tumor\textsuperscript{4}. The HylaPlat\textsuperscript{TM} reduced the C\textsubscript{max} 5.5 fold, indicating reduced toxicity of HylaPlat\textsuperscript{TM}\textsuperscript{5}.

Figure 2. Pharmacokinetics of HylaPlat in dogs.

A single injection of HylaPlat\textsuperscript{TM} resulted in size reduction of primary tumors in canines with squamous cell carcinomas or oral melanoma. At the completion of 3 treatments, two oral squamous cell carcinomas were completely eradicated and swelling was greatly reduced. Partial response or stable disease was reported in most of the relatively healthy tumor-bearing dogs.

Figure 3. A dog with spontaneous oral melanoma was injected intra-tumorally with 1 mg/kg HylaPlat. Seven days after a single HylaPlat administration, the tumor burden was significantly reduced. Cancer recurrence was not seen 1 year after treatment.

CONCLUSION
This study demonstrates that subcutaneous delivery of HylaPlat\textsuperscript{TM} formulations may be a promising treatment regimen to improve tumor drug accumulation and therapeutic efficacy. The successful completion of our large animal testing may provide a platform for the development of other polymeric drug formulations for the treatment of a wide spectrum of lymphatically metastatic cancers both in men and companion animals.

The HylaPlat formulation can either be administered as a neoadjuvant therapy prior to surgery to reduce tumor size and control cancer progression, or given as an adjuvant therapy post-surgery to reduce the risk of recurrence and eradicate cancer residuals, such as micrometastases in draining lymph nodes.

In addition, due to the sustained release characteristics of the conjugate, biweekly conjugate injections could replace the conventional daily infusion, leading to improved patient compliance and reduced healthcare cost. Approval of our IND application will warrant our human phase I clinical trial starting in 2015 and a phase IIa clinical trial starting in 2016.

REFERENCES

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