PEGylated Recombinant Human Hyaluronidase (PEGPH20): transition from a novel functional excipient to an API to increase the chemotherapeutic effectiveness against hyaluronan-rich cancers.

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Editorial

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The hyaluronidases are a family of enzymes that catalyze the hydrolysis of high molecular weight native hyaluronan or hyaluronic acid (HMHA), a constituent of the extracellular matrix (ECM), into low molecular weight hyaluronan (LMHA) fragments or oligomers. When administered intravenously (IV), recombinant hyaluronidase (rHuPH20) has a half-life in the order of minutes, which can be increased to ~20 hours when PEGylated (PEGPH20).

rHuPH20 has been approved as an excipient in subcutaneously (SC) administered immunoglobulin, rituximab and trastuzumab. rHuPH20 reversibly breaks down HA and allows for significantly larger volumes of these drugs (up to 700 mL) to be administered SC, thus facilitating a change over from the invasive IV to a more benign SC route. The SC route requires a lesser administration time, lesser frequency of administration and is more convenient for the patient. At the dosage administered SC, no measurable systemic concentrations of rHuPH20 were detected. Consequently, carcinogenicity studies have not been reported with rHuPH20 in accordance with ICH guideline S6 (R1). It is interesting to note that the carcinogenic potential of rHuPH20, or its PEGylated API may never be realized in healthy volunteers since its hypothesized metastatic effect requires pre-existing tumors.

Significant reduction in tumor interstitial fluid pressure (IFP) in animal models using IV administration of hyaluronidase has been reported in the literature. This reduction in IFP is due to a reduction of viscosity of the ECM, in turn caused by the hydrolysis of ECM HA by the enzyme. Consequently, PEGPH20, a PEGylated analog of the excipient rHuPH20, is being studied as a drug administered as an IV infusion (typically at a concentration of 3 μg/kg) in conjunction with gold standard chemotherapeutic agents to improve the delivery of the latter into HA-rich solid tumors. This is yet another instance of an excipient coming-of-age as an API, validating the paradigm of 'bioactive excipients' or excipients with innate pharmacological properties, that this author has long promulgated.

An enzyme that causes a decrease in the viscosity of the extracellular matrix surrounding the tumor tissue and a decrease in tumor IFP so as to promote passage of chemotherapeutic agents to the tumor tissue (breaching the castle walls) must also necessarily increase the probability of the escape and migration of malignant cells from the primary tumor site and

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promote metastasis (opening the flood gates). This logic, although self-evident, has never been empirically researched and only sporadically been explicitly stated as such. The author of a manuscript published in 1975 states "…Should it turn out that hyaluronidase does widen intercellular tumor spaces to the point of allowing entry of RE-cells, might it not, at the same time, have the effect of detaching clumps of malignant cells and actually predisposing to metastasis formation? - even though, one hopes, these cells would be again attacked at their new site(s) of adoption….".

HYAL1 and HYAL2 are the predominant isoforms of hyaluronidase functioning to catabolize HA in somatic tissues, predominantly into tetrasaccharide units. It has been suggested that increased levels of the isoform PH20 in mammary tissue may contribute to early invasion and metastasis of breast cancer. HYAL1 was found to be an independent prognostic indicator of prostate and bladder cancer

Significant ambiguity exists in the literature as regards the tumor promoting or tumor suppressing properties of hyaluronidase, as well as, those of the LMHA fragments or oligomers that the enzyme generates, upon hydrolysis of HMHA. The inflammation and tumor promoting results have been attributed to contamination with endotoxins and peptidoglycans in commercially available hyaluronidase reagents or in HA fragments. However, it appears that investigators have been aware of these confounding factors and have explicitly either corrected or tested for them as evident from the literature. Moreover, studies involving knockdown or knockout gene models incorporate HA fragments or hyaluronidase into both control and test protocols. Furthermore, some investigators have used HYAL-1 or HYAL-3 cDNA constructs to transfect cells of interest or HAS genes that perturb HA synthesis (rather than using hyaluronidase to modulate HA degradation) that have no chance of endotoxin contamination. Endotoxin independent binding of HA to cell surface receptors such as CD44 has been shown to activate downstream signaling pathways such as MAPK, Rac, and PI3K leading to cell survival, proliferation, migration and invasion. Lastly, some studies have only monitored relative expression levels of HA or associated enzymes (including hyaluronidase) without any extraneous addition. Although not all manuscripts address inflammation *per se*, different outcomes have been observed with regard to cell adhesion, motility, invasiveness and progression with different levels of hyaluronidase expression and different molecular weights of HA. Taken together, it is therefore more likely that the tumor promoting properties of hyaluronidase and the observed differences between HMWH and LMWH are real, at least in *in vitro* animal models under cell-specific conditions, which cannot all be attributed to artifacts caused by adventitious impurities or to differences in research methodology.

These concerns about the tumor or metastasis promoting effects of hyaluronidase can be compared with current chemo or radiotherapy, which significantly increases mutagenic risk and subsequent carcinogenesis. Furthermore, such is the dismal survival rate for Pancreatic ductal adenocarcinoma (PDAC), one of the type of solid tumors in which PEGPH20 is being tested in clinical trials, that a significant increase in objective survival (OS) may still not be enough for any hypothesized metastasis to grow sufficiently before death occurs. Put differently, the patient may not live long enough, even after a significant improvement in OS, to realize the speculative carcinogenic potential of PEGPH20. PEGPH20 is also being studied in other types of cancers in conjunction with checkpoint inhibitor APIs'. If OS in these cancers shows marked improvement, the risk of death from subsequent carcinogenesis would be inconsequential to regulatory approval due to a similar risk existing with current chemotherapy gold standard treatment.

rHuPH20 is a novel, new functional excipient that can increase SC drug volume, thus facilitating a change over from the invasive IV to the more benign SC route. Its PEGylated analog, PEGPH20, using the same mechanism of action as rHuPH20, has shown, and continues to show, promising results when combined and administered intravenously together with chemotherapeutic agents, in treating tumors

characterized by high levels of HA in their ECM. However, its non-mutagenic mechanism of action incorporates a hydrodynamic attribute (of reduced viscosity and increased perfusion in the tumor vicinity) combined with the generation of LMHA oligomers, both of which are amenable toward facilitating the metastasis of the existing tumor. This is no different from current chemotherapeutic or radiation therapy regimens where the drugs/radiation itself is not entirely devoid of mutagenic effects and carcinogenesis. Judiciously used, the PEGylated excipient rHuPH20 has the ability to significantly prolong OS in HA-rich tumors, thus making it yet another excipient-to-drug transition instance in an ever expanding armamentarium of 'bioactive excipients'.