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Intratumoral generation of 2-fluoroadenine to treat solid malignancies of the head and neck.

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Abstract

This report describes treatment of locoregional head and neck squamous cell carcinoma (HNSCC) by an innovative, experimental strategy involving generation of a robust anti-cancer agent (2-fluoroadenine; F-Ade) following transduction by *E. coli* purine nucleoside phosphorylase (PNP) in a small number of tumor cells. F-Ade works by a unique mechanism of action (ablation of RNA and protein synthesis) and confers tumor regressions of otherwise refractory HNSCC in human subjects. Clinical studies have now advanced to a pivotal (registration-directed) trial involving locoregional HNSCC, with plans to begin subject enrollment late in 2018. The present review is the first to summarize use of PNP in the context of HNSCC, and provides background regarding this emerging anti-cancer approach.

Keywords

HNSCC; 2-fluoroadenine; gene transfer; purine nucleoside phosphorylase; clinical trial

Gene-directed enzyme prodrug therapy in HNSCC

Because of the continuing need for more effective and well tolerated therapies, locoregional head and neck squamous cell carcinoma has been viewed as an optimal target for “proof of concept” regarding gene-based prodrug activation. End-stage tumors of the head and neck have few treatment options and are associated with severe morbidity, including uncontrolled bleeding, chronic pain, infection, aspiration, poor airway control, and vocal cord

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Conflict of Interest

Drs. Parker and Sorscher have ownership interests in PNP Therapeutics and serve on the Board of Directors for the company, which develops products used in research described by this paper. Drs. Parker and Sorscher are also inventors of technology being evaluated in studies described by this report. The terms of this arrangement for Dr. Sorscher have been reviewed and approved by Emory University in accordance with its conflict of interest policies.

impairment, among others ¹. In addition, use of chemotherapy or external beam radiation therapy to treat end stage HNSCC is associated with severe clinical toxicity. These features indicate the potential usefulness of a locally administered anti-tumor agent, and numerous clinical studies evaluating safety and efficacy of experimental, gene-based therapeutics to evaluate cyto reduction have been conducted in HNSCC ²⁻⁵.

Gene based prodrug activation

Expression of an activating gene intratumorally - followed by systemic prodrug treatment - can be used to generate high concentrations of active chemotherapy within a malignant mass. Tumor-directed expression of cytosine deaminase (CD), for example, has been applied to produce 5-fluorouracil (5-FU) from 5-fluorocytosine ⁶, and herpes simplex virus thymidine kinase (HSV-tk) expressed intratumorally has been utilized for producing ganciclovir nucleotides from systemically administered ganciclovir ⁷. Very early prodrug activation systems such as these have not been successful in the clinic for a number of reasons. First, it is unclear whether drug concentrations or dose intensity of compounds elaborated in this manner are above what can be achieved with systemic or regional administration (e.g., using a compound such as 5-FU).

Second, 5-FU and ganciclovir triphosphate are designed to disrupt DNA synthesis and specifically impair the rapidly dividing cell compartment within tumors such as HNSCC. The strategy is therefore limited by low growth fraction of head and neck cancer, in which proliferative index is typically less than 20% of a malignant mass at any point in time ⁸. Even if numbers of dividing head and neck tumor cells were as high as 50% (and even if a cumulative exposure to an activated prodrug were able to destroy all mitotically active tumor cells), a treated tumor under these conditions would still be only one doubling time away from regaining pre-treatment dimensions.

Moreover, compounds such as ganciclovir nucleotides partition inefficiently across cell membranes, although they may be transmitted within certain cancers by gap junctions or other cell-to-cell communication pathways. Membrane impermeant drugs are primarily active against cells in which they are generated, and current gene delivery vehicles are typically inefficient, with *in vivo* tumor transduction efficiencies substantially below 5% of target cells. As a result, agents produced by these previous technologies have shown minimal effect in patient-oriented studies.

Clinical data resulting from gene-based cyto reduction of HNSCC

Gene-directed enzyme/prodrug treatment for HNSCC can be categorized as 'corrective' gene therapy, which aims to inhibit tumor oncogenes or alter tumor suppressor genes, and/or 'cyto reductive' gene therapy, in which HNSCC cells are killed directly. More than 250 cellular genes have been suggested to contribute during pathogenesis of HNSCC ⁹. Targeting the p53 tumor suppressor has been commercially marketed as a corrective gene therapy (Gendicine, Schenzhen SiBono GenTech) ¹⁰. Gendicine has been reported to confer meaningful response rates as a single agent, as well as improved outcomes in patients with advanced HNSCC in combination with radiation treatment or conventional chemotherapy.

An earlier approach to target p53 utilized conditionally replicating adenovirus capable of binding and repressing the oncogene. One such example was ONYX-015, which conferred tumor regression in 10% of patients in phase II trials with an acceptable safety profile ¹¹.

Generation of purine base chemotherapy within tumor parenchyma

In contrast to production of compounds such as ganciclovir triphosphate or 5-FU by prodrug metabolism in a tumor mass, adenine analogs such as 6-methylpurine or 2-fluoroadenine (F-Ade) are more potent, rapidly ablate the non-dividing tumor cell compartment and freely partition between and among tumor cells ¹². Agents such as these primarily interrupt RNA and protein synthesis (as well as DNA replication) and are therefore active against low growth fraction tumors such as HNSCC (and expected to destroy tumor progenitor cells and so-called “giant” tumor cells). F-Ade, for example, disrupts crucial pathways required for cell viability irrespective of proliferative status (i.e. cycling or quiescent). Moreover, F-Ade is much more potent than 5-FU¹³. Intratumoral (and intracellular) production of F-Ade elicits pronounced tumor involution *in vivo*, and its subsequent release into systemic circulation results in plasma concentrations orders of magnitude lower than in the tumor, itself. In addition, F-Ade is systemically inactivated by the ubiquitous mammalian enzyme, xanthine oxidase. *In vivo* studies to test clearance of F-Ade released from tumor parenchyma have shown serum levels that are undetectable in animal models and human subjects following PNP-based treatment of HNSCC, despite substantial antitumor activity ¹⁴.

Mechanisms for purine based generation within a tumor mass *in vivo*.

Nucleosides such as fludarabine, 6-methylpurine-2'-deoxyriboside, and 2-fluoro-2'-deoxyadenosine are efficiently hydrolyzed by prokaryotic purine nucleoside phosphorylase enzymes from numerous species (Figure 1). The same agents are poor substrates for mammalian PNP ¹⁵. Eukaryotic PNP assembles as a homotrimer, whereas PNP from bacteria (such as *E. coli*) is homo-hexameric and includes an attack group within the active site that facilitates cleavage of adenine-based nucleosides ¹⁶. Expression of *E. coli* PNP in a small number of tumor cells (e.g. 2.5%) mediates pronounced tumor regressions in animal models, and similar effects have been demonstrated in phase I human clinical studies ^{14,17,18}. Other prokaryotic PNPs, including the *T. vaginalis* enzyme, generate F-Ade from fludarabine with Vmax/Km 20–25 fold higher than *E. coli* PNP, and represent an important future means of improving the technology. Generation of anticancer compounds using these enzymes is most effective intracellularly. Tumor half-life of nucleotides generated from highly active purine bases liberated by PNP-mediated hydrolysis is more than 12 hours *in vivo*, whereas simply injecting a purine base into the extracellular space of a tumor mass confers half-life in tumor tissue of less than ten minutes. This is in part due to retrograde release of inoculated agents through the needle track, rapid escape to systemic circulation following intratumoral dosing, extracellular metabolism of F-Ade, or other factors ^{19,20}.

Direct comparisons of anticancer activity using HSV-tk or CD versus purine base generation (e.g. F-Ade) have been performed *in vitro* and *in vivo*. Nestler and colleagues, for example, applied a foamy virus delivery vehicle and compared efficiency of PNP to HSV-tk or CD in fibrosarcoma, glioblastoma and hepatoma cell lines. Target cells transduced with a reporter

gene and parental (non-transduced) cells were tested as controls. The PNP approach killed the malignant cells in a dose dependent fashion more rapidly and completely than HSV-tk or CD, and much more effectively *in vitro*²¹. Lockett et al. demonstrated that *E. coli* PNP expression elicited profound killing of human prostate (PC-3) and breast cancer (MCF-7, T47-D2) cell models²². In other studies, adenoviral vectors encoding HSV-tk or *E. coli* PNP were tested in parallel. Prostate cancer cells were resistant to ablation by HSV-tk (multiplicity of infection (MOI) at 10, 20, 50, or 100 conferred less than 50% inhibition of growth by ganciclovir), while the PNP gene killed 100% of the cancer cells at every MOI evaluated. Breast carcinoma *in vitro* was strongly resistant to HSV-tk/ganciclovir at all MOI's, but cells were completely ablated by the PNP/nucleoside combination. In another study of prostate cancer, Xie et al. showed that *E. coli* PNP mediated 100% cell killing in LnCaP cells, whereas HSV-tk expression and GCV led to substantially less cell death²³. When adenovirus encoding *E. coli* PNP/fludarabine or HSV-tk/ganciclovir were examined for regression of prostate xenografts in vivo, PNP led to more robust anti-tumor activity as well as 20% long term survivors (>450 days, compared to no long term survivors following HSV-tk/ganciclovir)²⁴.

Clinical evaluation of the PNP technology

Based on extensive *in vivo* analysis of human tumor xenografts and other cancer models demonstrating robust suppression of human lung, breast, melanoma, prostate, hepatic, glioma, head/neck, and other tumors in mice^{25–32} and unpublished results, a phase I clinical trial was conducted to evaluate intratumoral generation of F-Ade¹⁴. Replication deficient (first generation) E1 and E3 deleted adenovirus encoding *E. coli* PNP was inoculated along multiple needle tracks into HNSCC or other locoregional tumor masses, followed by systemic administration of fludarabine phosphate at doses lower than those approved clinically for treatment of chronic lymphocytic leukemia. Subsequent to blood stream dephosphorylation of prodrug, fludarabine is cleaved intratumorally to generate F-Ade (Figure 1), leading to dose-dependent tumor regressions in otherwise untreatable HNSCC. Malignant tissue in the same patients given only fludarabine phosphate (but not adenovirus encoding PNP), was used to establish specificity¹⁴.

The approach was safe and well tolerated, without serious adverse events or dose-limiting toxicity. Greater than 60% overall response rate was obtained in a small number of patients at higher dosing cohorts, and since the best available interventions for end-stage HNSCC have considerably lower rates of tumor regression (e.g. checkpoint blockade inhibitors exhibit overall response rates less than 25% in this setting)³³, FDA has indicated willingness to consider registration if comparably high response rates can be obtained as part of a follow-on, single-arm study of individuals with locoregional (needle accessible) HNSCC. This clinical indication has received orphan drug status from FDA, and a pivotal, patient oriented trial was recently approved by the agency, with enrollment planned to begin in the fourth quarter of 2018. The phase 1/2 trial is designed to evaluate: 1) safety and efficacy of the approach with repeat cycles, and 2) clinical response when the entire tumor burden from a cohort of HNSCC patients is treated. Subjects will receive 2×10^{11} Ad/PNP viral particles per dose, given 3 times over days 1 and 2, followed by fludarabine phosphate, 25 mg/m², administered on days 3–5. Repeat dosing of the 5-day cycle of Ad/PNP plus

fludarabine phosphate will be performed every 4 weeks until no tumor is accessible for injection (maximum of 5 cycles). The primary efficacy endpoint for the phase 1/2 study will be overall response rate.

PNP for treatment of metastatic HNSCC tumors

Technologies designed to route therapeutic genes to metastatic tumors *in vivo* have advanced dramatically over the past few years, and include new viral constructs with cancer tropism, lipid nanoparticles (LNPs) identified by unbiased chemical library screening, ultrasound activated vehicles, tumor infiltrating lymphocytes, exosomes, and nanocage constructs, among others^{34–42}. Based on extensive preclinical findings, purine nucleoside phosphorylase represents an excellent “payload” for vectors of this type—particularly since intratumoral generation of highly potent drugs such as F-Ade mediate antitumor activity when a very small fraction of cells in the tumor mass (e.g., < 1–3%) have been transduced¹⁷.

Combination of PNP treatment with other modalities

Based on mechanism of action fundamentally different from all approved anticancer agents (as well as all experimental antitumor strategies), it is anticipated that PNP-based therapy will exhibit additivity or synergy with existing cancer modalities. This has been formally shown for combination treatment of PNP/fludarabine with external beam radiation *in vivo* against human tumor xenografts in mice¹⁸. In addition, anticancer immune stimulation by intratumoral F-Ade has been demonstrated previously in murine models of prostate cancer⁴³. Because increasing evidence suggests that many approaches to ablating tumor tissue may also enhance immune clearance, it is reasonable to imagine F-Ade will act to augment immune-type treatments such as those that influence checkpoint blockade. This assertion is currently being tested in preclinical animal models *in vivo*.

Concluding statements/ future directions

In summary, intratumoral generation of purine base chemotherapy offers a powerful approach to HNSCC cytoreduction. The strategy has already shown promise in locally advanced, regional head/neck cancers in human subjects, and a pivotal trial targeting HNSCC has received FDA approval, with plans to enroll patients in the fourth quarter of 2018. No other cancer treatment to date has mediated generation of such highly active drugs specifically and safely within tumor tissue, and one can argue that an approach of this type (which has been shown safe in phase I clinical studies) may be necessary to address refractory, low growth fraction human tumors such as HNSCC. Limitations of the technology include restriction in current form to disease without distant metastases, although delivery vehicles continue to emerge that may be suitable for addressing disseminated head and neck cancer. Purine nucleoside phosphorylase enzymes with enhanced nucleoside cleavage kinetics, improved prodrug delivery by intratumoral administration¹⁸, and new compounds that more efficiently liberate novel and potent intratumoral chemotherapy, represent important areas under development for the future.

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Figure (1):
Conversion of Fludarabine phosphate to F-Ade.