Phase 1/ 2 study of Ad/PNP with fludarabine for the treatment of head & neck squamous cell carcinoma

A. Dimitrios Colevas[1*], Eric J. Sorscher[2*], William Parker[3], Roan Courtney Raymundo[1], Jeong S. Hong[2], Regina Rab[2], Camilo Henao[4], Nikki Schmitt[2], Madison Stallings[2], Kelly T. McKee, Jr.[5], Eben Rosenthal[6], Joseph Curry[4]

1. Stanford Cancer Institute, Stanford University; 2. Emory University School of Medicine; 3. PNP Therapeutics, Inc.; 4. Thomas Jefferson University; 5. GeoVax Laboratories; 6. Vanderbilt University

Background

We are evaluating intratumoral nucleoside cleavage by *E. coli* purine nucleoside phosphorylase (PNP) as an experimental therapy for refractory solid tumors. The approach requires delivery of PNP transgene to tumor parenchyma followed by prodrug administration and provides "bystander" killing by a very potent purine antimetabolite (F-Ade) generated intratumorally. F-Ade is 1,000-times more active than fluorouracil, the chemotherapeutic produced by cytosine deaminase (CD; a first-generation construct used for tumor sensitization). PNP/F-Ade has been found superior to CD/fluorouracil by several laboratories. In a Phase 1 study (Rosenthal *et al., Ann. Oncol.*), antitumor activity was observed following IT injections of a recombinant adenovirus encoding PNP (Ad/PNP), followed by IV fludarabine phosphate (F-araAMP, a prodrug converted by PNP to F-Ade).

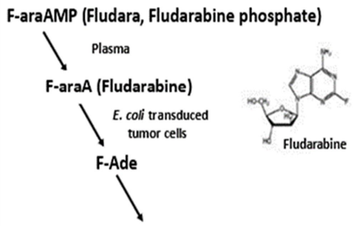

Figure 1. Prodrug activation by *E. coli* PNP. Fludarabine is cleaved to liberate F-Ade, a compound that disrupts DNA, RNA, and protein synthesis.

Robust and dose dependent tumor regressions in animal models using a PNP based approach

Figure 2. The anti-tumor effect of F-araAMP exhibits dose dependence on the level of prodrug administered. D54 glioma tumors were established from an inoculum in which 10% of cells stably expressed *E. coli* PNP. Anti-tumor efficacy was greater at 100 mg/kg F-araAMP than at 50 mg/kg or 25 mg/kg given over a standard 3-day schedule. F-araAMP treatment groups were significantly different from non- treatment group ($p<0.001$).

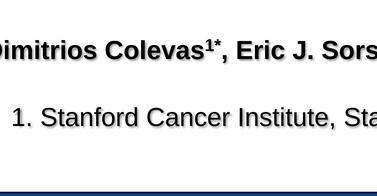

Figure 3. Tumor regression using *E. coli* PNP and F-araAMP exhibits dose dependence on the level of transgene expression. D54 glioma tumors were established with decreasing proportions of PNP expressing cells and parental cells. F-araAMP was 100 mg/kg q2h x 5, q1d x 3 days beginning on Day 14. F-araAMP treatment groups were significantly different from non-treatment groups ($p<0.001$).

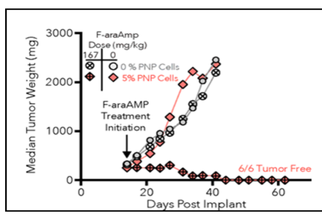

Figure 4. Modified F-araAMP schedule in D54 tumors that express *E. coli* PNP in ~5% of the cells. Parental D54 tumor cells were mixed with D54 tumor cells stably transduced with *E. coli* PNP so that ~5% of the mixture expressed the PNP transgene. Intraperitoneal treatment with F-araAMP (167 mg/kg 3 times per day for 3 consecutive days, or vehicle control) began on day 14. The effect of this F-araAMP schedule on parental D54 tumors is also shown, indicating that F- araAMP is not active against tumors that do not express *E. coli* PNP. Tumor growth in the F-araAMP treatment group with PNP expression was significantly different than that in vehicle treated or parental controls ($p < 0.001$).

Phase I Clinical Trial

A protocol was developed to test *E. coli* PNP in 12 human subjects (10 subjects with head and neck cancer and 2 subjects with melanoma - 9 men and 3 women) with otherwise untreatable solid tumors. The study was reviewed by FDA (CBER/OCTGT) and conducted under IND 14271. Ad/PNP was administered IT, followed by systemic dosing of F-araAMP. This trial was the first to evaluate the anticancer purine base F- Ade in human subjects. The overall response rate (complete response + partial response) was 66.7% in the two highest dose cohorts (Cohorts 3 and 4), with a more modest response in Cohort 2 and no antitumor activity in Cohort 1.

Cohort	Total Ad/PNP	Total F-araAMP (regimen)
1	3 x 10^{11} VP (1 x 10^{11} VP x 3 injections)	15 mg/m^2 (5 mg/m^2 daily for 3 days)
2	3 x 10^{11} VP (1 x 10^{11} VP x 3 injections)	45 mg/m^2 (15 mg/m^2 daily for 3 days)
3	3 x 10^{11} VP (1 x 10^{11} VP x 3 injections)	75 mg/m^2 (25 mg/m^2 daily for 3 days)
4	3 x 10^{12} VP (1 x 10^{12} VP x 3 injections)	75 mg/m^2 (25 mg/m^2 daily for 3 days)

Table 1. Schematic of Phase I Clinical Trial

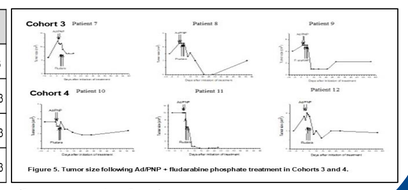

Figure 5. Tumor size following Ad/PNP + fludarabine phosphate treatment in Cohorts 3 and 4.

(Rosenthal et al., Ann. Oncol.)

Method

Patients in the present Phase 1 / 2 trial have RECIST 1.1 measurable tumors amenable to local injection and no other palliative treatment options. A single-arm protocol is being used to evaluate safety of repeat cycles of Ad/PNP and F-araAMP. Ad/PNP (Gedeptin™) is injected intratumorally twice on Day 1 and once on Day 2, followed by infusion of F-araAMP on Days 3, 4, and 5 every 4 weeks for up to 5 cycles.

Progress to date

Patient	Gender	Ethnicity	Age (year)	Tumor type	Number of cycles	Target lesion response
201-001	Female	Asian	70	HNSCC	1	PD
201-002	Male	Asian	52	HNSCC	1	SD
201-003	Male	White	47	LEC[1]	5	SD
201-004	Male	Asian	53	NPC[2]	3	SD
201-005	Male	Asian	58	HNSCC	1	SD
202-001	Male	Asian	58	HNSCC	4	SD
203-001	Male	White	66	HNSCC	1	PD
203-002	Female	White	66	HNSCC	1	N/A

Table 2. Demographic and treatment related data in individual patients. [1] Lympho-epithelial carcinoma, [2] Nasopharyngeal carcinoma.

Criteria	Description
Complete Response (CR)	Disappearance of all target lesions
Partial Response (PR)	At least a 30% decrease in the sum of the LD of target lesions, taking as reference the baseline sum LD
Progressive Disease (PD)	At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions
Stable Disease (SD)	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started

Table 3. Recist 1.1 criteria for evaluation of target lesions

Patient	Total Ad/PNP/cycle	Total Fludarabine/cycle
201-001	5 x 10^11 VP (1x10^11 x 1 injection + 2x10^11 x 2 injections)	75 mg/m^2 (25 mg/m^2 daily for 3 days)
201-002	6 x 10^11 VP (2x10^11 x 3 injections)	75 mg/m^2 (25 mg/m^2 daily for 3 days)
201-003	6 x 10^11 VP (2x10^11 x 3 injections)	75 mg/m^2 (25 mg/m^2 daily for 3 days)
201-004	6 x 10^11 VP (2x10^11 x 3 injections)	75 mg/m^2 (25 mg/m^2 daily for 3 days)
201-005	6 x 10^11 VP (2x10^11 x 3 injections)	75 mg/m^2 (25 mg/m^2 daily for 3 days)
202-001	6 x 10^11 VP (2x10^11 x 3 injections)	75 mg/m^2 (25 mg/m^2 daily for 3 days)
203-001	6 x 10^11 VP (2x10^11 x 3 injections)	75 mg/m^2 (25 mg/m^2 daily for 3 days)
203-002	5 x 10^11 VP (1x10^11 x 1 injection + 2x10^11 x 2 injections)	75 mg/m^2 (25 mg/m^2 daily for 3 days)

Table 4. Schematic of Ad/PNP and Fludarabine treatment for individual patients

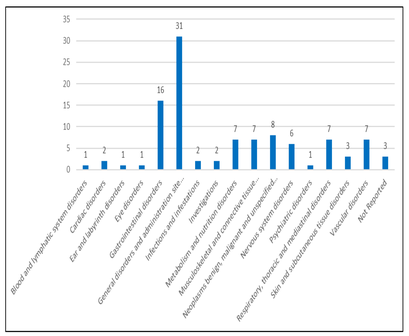

Figure 6. Adverse events by SOC (System Organ Class)

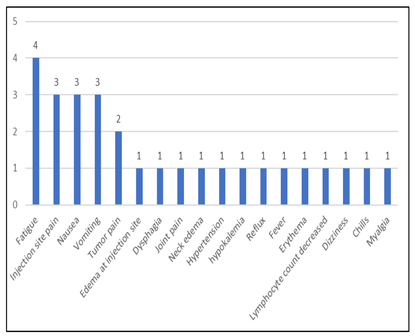

Figure 7. Adverse events attributable to study drug

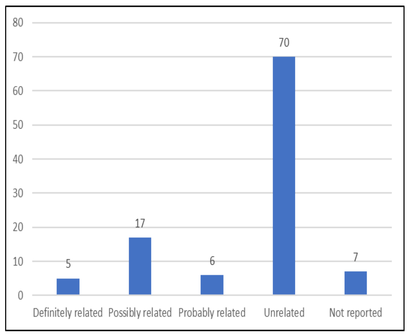

Figure 8. Adverse events by relationship to the drug

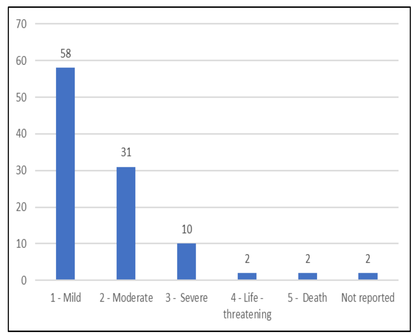

Figure 9. Adverse events by grade. Two cases of death and the 2 cases of life-threatening events were unlikely related to treatment.

Study Progress

1. Findings are from an ongoing clinical study and data cleaning / final analysis are needed to evaluate the safety and efficacy of Ad/PNP.
2. Ad/PNP detection (E1a and PNP) by PCR has been completed for the first 5 study subjects using whole blood and urine samples from enrolled individuals.
   - E.coli PNP gene was detected in all blood samples following Ad-PNP injection, but not prior to Ad-PNP dose.
   - E1a positive, replication competent viruses was not detected in any of the blood or urine samples.
   - E.coli PNP gene was detected in urine samples from a subset of patients.
3. Low-level F-Ade was noted in blood samples following PNP therapy in certain subjects.
4. DNA and mRNA protocols (for testing PNP delivery and transgene expression, respectively) have been completed for the first 5 study subjects using tumor tissue.
   - PNP gene delivery was detected in all tumor samples and every tumor section.
   - Expression of PNP gene was detected in all samples except one – this sample was particularly small and there was not sufficient tissue to repeat the experiment.
5. Serum antibody titers against adenovirus are being determined for study subjects enrolled to date.
6. Histopathology of over 600 tumor sections is being assessed using 22 immunological markers as part of tissue and mechanistic analysis.
7. Findings from Patients #6 - 8 continue to be acquired.

Ad/PNP Insights

- Evidence of antitumor activity: Stable disease noted in 5 of 8 patients in treated tumors.
- In the current Phase 1 / 2 trial, there have not been any dose limiting toxicities or serious adverse events (SAEs) definitively attributable to treatment.
- One patient with HNSCC has demonstrated tolerability of 5 treatment cycles without limiting sequelae.
- The strategy is also being considered for earlier-stage HNSCC with less tumor burden, including a role similar to neoadjuvant or cytoreductive radiotherapy in combination with checkpoint blockade inhibition.
- A muti-center trial is planned to define MTD and feasibility in smaller tumors.
- Challenges:
  - Regressions of large tumors remains a challenge, likely due to the low percentages of PNP transduced cells achieved with small volume Ad/PNP injections.
  - Acute swelling of tumor tissue following intratumoral virus injection has been observed in two patients, consistent with inflammatory response and/or disease progression.

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