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Interferon-γ (IFN-γ) response correlates with survival in a Phase 2 Hyperacute (tergenpumatucel-L) immunotherapy trial for non-small cell lung cancer (NSCLC)

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Background

Non-small cell lung cancer (NSCLC) remains the leading cause of cancer death with an overall 5-year survival of less than 15%.

We studied the safety and activity of a novel immunotherapy that utilizes human lung cancer cell lines engineered to express α(1,3)galactosylepitopes (tergenpumatucel-L)

HyperAcute-Lung (tergenpumatucel-L) Immunotherapy

- A major barrier to xenotransplantation from lower mammals into primates or humans is immunity to α(1,3)galactosyl epitopes expressed on the tissues of lower mammals that induce a phenomenon known as hyperacute rejection (Fig. 1)[1,2]
- α(1,3)galactosyltransferase (αGT) is expressed by lower mammals, but not in humans or other Old World primates.
- Humans have naturally induced complement-fixing anti-αGal antibodies (Ab) at levels that can reach 1-2% of total circulating antibodies.
- When αGal-positive cells are exposed to primate sera, anti-αGal antibodies bind the αGal epitopes leading to rapid activation of complement and cell lysis. This process is the underlying mechanism of hyperacute rejection.
- Whole cell tumor vaccines expressing αGal epitopes are significantly more effective than their αGal-negative counterparts as immunotherapy against established tumors in animal models[3,4,5,6]
- Tergenpumatucel-L immunotherapy consists of stably transduced human lung cancer cell lines (HAL1, HAL2 and HAL3) expressing the murine αGT gene (Fig. 2)

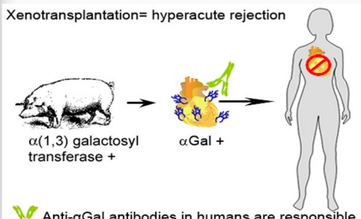

Figure 1. Hyperacute rejection and immunotherapeutic applications.

Hyperacute rejection of a xenotransplant occurs due to the presence of cell surface α(1,3) galactosyl residues in tissues expressing the αGT enzyme. These epitopes are highly immunogenic in man[2] and make lung cancer cell lines bearing the epitopes susceptible to rapid lysis when exposed to human sera.

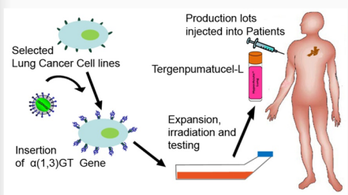

Figure 2. Tergenpumatucel-L immunotherapy.

Tergenpumatucel-L immunotherapy is comprised of three human lung cancer cell lines transduced with a retroviral vector expressing the murine αGT gene (HAL1, HAL and HAL3). Product is irradiated for safety and administered as a series of intradermal injections.

Objectives

Primary Objectives:

- Phase I: Determine the adverse events, dose-limiting toxicity and safety of tergenpumatucel-L immunotherapy in patients with advanced NSCLC.
- Phase II: Determine survival benefit of tergenpumatucel-L immunotherapy in patients with advanced NSCLC who had previously failed 1st line chemotherapy..

Secondary Objectives:

- Assess survival distribution and duration of responses.
- Evaluate correlative immunological studies including:
  - Measure interferon-gamma (IFN-γ) and interleukin-5 (IL-5) responses to tergenpumatucel-L immunotherapy using whole-cell ELISPOT assay
  - Effect of tergenpumatucel-L immunotherapy on serum anti-αGal and anti-CEA Ab.

Methods

Clinical Protocol and Sample collection

- In Phase I, 5 dose cohorts received increasing amounts of HyperAcute-Lung once a month up to 4 doses, escalating from 3 million cells to 300 million cells.
- In Phase II, all patients received 300 million cells/injection every 2 weeks for up to eight scheduled doses
- Serum samples were collected prior to immunization, on days 29, 57, 85, and 127 post-immunization, and then at two month follow up visits. Anti-CEA Ab and anti-αGal Ab were determined by ELISA.
- PBMC were collected prior to immunization, after the 4th vaccination and after the 8th vaccinations (Phase II).

Results

Safety data

- The most frequent adverse event attributed to the vaccine was skin reactions at the site of immunization

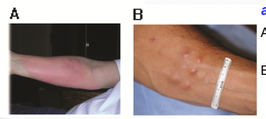

Figure 3. Skin reactions frequently observed after tergenpumatucel-L immunotherapy.

A. Commonly observed rapid-acute inflammatory reaction at the site of immunization.

B. Delayed-type hypersensitivity (DTH)-like reaction that occurs days to weeks after immunization. These skin reactions resolve without intervention in the vast majority

Anti-CEA Ab response

- In Phase 1, 40% of tested patients responded with increased anti-CEA Ab (>20%, n=10)
- In Phase II, 71 % of tested patients responded with increased anti-CEA Ab (>20%, n=21)
- The differences in the response among Phase I and Phase II anti–CEA Ab reactivity is statistically significant suggesting a dose-response effect (chi-square $p<0.001$)

IFN-γ response.

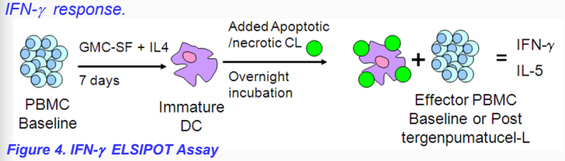

Figure 4. IFN-γ ELSIPOT Assay

IFN-γ production was measured by ELISPOT. Tumor-loaded autologous DC were cultured with effector PBMC before and after tergenpumatucel-L immunotherapy. The fold-increased (FI) in reactivity was calculated as FI = test/baseline. The cell lines used in this study included the un-modified parental cell lines of the three cellular components of tergenpumatucel-L and a fourth, unrelated lung adenocarcinoma that is not a product component.

IFN-γ response.

- 18 patients with paired pre-immunization and post-immunization samples were tested.
- 11 of 18 patients (61%) responded with increased IFN-γ after immunization.
- The OS of patients responding with increased IFN-γ was 21.9 months in comparison to 7.2 months for non-responders.
- A significant difference was demonstrated in the overall survival of patients responding with increased IFN-γ after immunization compared to non-responder patients (p=0.044).
- 6 of 11 (55%) responder patients showed reactivity to CL4-H522, a non related lung adenocarcinoma not component of tergenpumatucel-L , suggesting cross-priming to shared antigens.

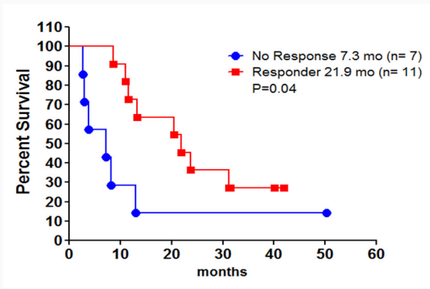

Figure 5. IFN-γ response after tergenpumatucel-L treatment.

Kaplan-Meier curve for the overall survival of responder and non-responder patients in the IFN-γ ELISPOT assay.
Responders were defined as those patients producing 10-fold or more increased IFN- γ response to the cell lines tested after vaccination with tergenpumatucel-L.
The difference was statistically significant (p=0.044)

Clinical response Phase II patients

- 28 patients were treated and evaluable for response.
- 8 (28.5 %) demonstrated stable disease (SD) ≥16 wks including one patient that initially progressed and later regressed, surviving over 40 months.
- Median overall survival (OS) was 11.3 months (95% CI 3.8-21.9) with 1-year OS of 46%.

			Serious Adverse Events (CTC Grade 3 or 4) Attributed to therapy			
Therapy	Median OS (Months)	1 Year Survival	Nausea	Fatigue	Anemia	Neutropenia
Best Supportive Care (7)	**4.6**	**11%**	**--**	**--**	**--**	**--**
Docetaxel (8)	**7.5**	**37%**	**1.8%**	**5.4%**	**4.3%**	**40%**
Pemetrexed (8)	**8.3**	**30%**	**2.6%**	**5.3%**	**4.2%**	**5.3%**
HyperAcute-Lung	**11.3**	**46%**	**0%**	**0%**	**0%**	**0%**

Table 1 . Efficacy and safety data comparison

Comparative results for second-line treatment in advanced NSCLC with pemetrexed, docetaxel and tergenpumatucel-L including safety data.

Conclusions

- Significantly longer overall survival (OS) is demonstrated in patients responding with increased IFN-γ secretion by PBMC's post-vaccination (21.9 vs. 7.2, p=0.044).
- Tergenpumatucel-L immunotherapy induced anti-tumor reactivity to the parental αGal negative cell lines as measured by IFN-γ response in 11 of 18 tested patients.
- Acquired reactivity to CL4-H522 cell line, not part of the tergenpumatucel-L vaccine, suggests antigen cross-priming to shared tumor antigens in lung cancer cell lines .
- A significant higher percentage of patients responded with anti-CEA Ab in Phase II compared to Phase I patients (71% vs. 40%, p<0.001) suggesting a dose-dependent response .
- The median OS was 11.3 months with 46% of patients surviving one year. This data compares favorably with historical data of second-line chemotherapy treatment for NSCLC suggesting encouraging clinical benefit for advanced NSCLC, particularly with the absence of significant serious adverse events associated with conventional cytotoxic chemotherapies.
- Immunological monitoring of patients enrolled in tergenpumatucel-L trials is feasible and may provide valuable correlative information to determine efficacy in this immunotherapy trial .

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