Correlation of Anti-calreticulin Antibody Titers with Improved Overall Survival an a Phase 2 Clinical Trial of Algenpantucel-L Immunotherapy for Patients with Resected Pancreatic Cancer

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INTRODUCTION

Algenpantucel-L immunotherapy consists of allogeneic pancreatic cancer cells that have been genetically modified to express the carbohydrate α(1,3)Gal, to which humans have an inherent pre-existing immunity. αGal is primarily responsible for the hyperacute rejection of foreign tissue via this potent immune defense mechanism in humans (1). Algenpantucel-L leverages this hyperacute rejection mechanism to educate the immune system towards components of the patients' own tumor cells (2).

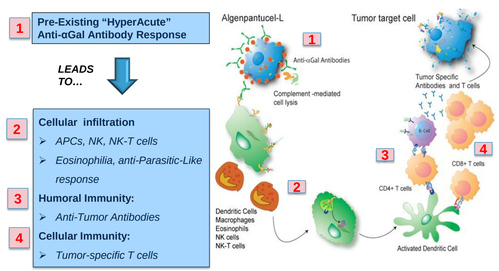
Calreticulin (CALR) is a calcium-binding chaperone protein that functions in the immune response by folding major histocompatibility complex (MHC) class I molecules and influencing antigen presentation to cytotoxic T cells. In pre-clinical models, drugs that induce cell surface CALR confer enhanced anti-tumor response (3-5). Components of algenpantucel-L express cell surface CALR.

This open label, multicenter Phase 2 study (NLG205) is designed to evaluate algenpantucel-L plus standard of care gemcitabine with 5-FU-XRT for resected pancreatic cancer.

ALGENPANTUCEL-L: PROPOSED MOA

- Algenpantucel-L consists of tumor-specific human cancer cell lines genetically altered to express a unique carbohydrate, **α-gal**
- Humans have pre-existing immune response to **α-gal**
- Algenpantucel-L is an allogeneic whole-cell vaccine that utilizes this potent, pre-existing immune response against α-gal to educate the immune system and attack cancer

Figure 1: Algenpantucel-L "Hyperacute" Immunotherapy Proposed Mechanism



ALGENPANTUCEL-L: POTENTIAL CRITICAL SUCCESS FACTORS

Formulation

- Metabolically active whole cell vaccine
- Expression of polyvalent tumor antigens
- Presence of tumor antigens shared with patient's cancer
- Not patient specific, adaptable logistics and manufacture

Mechanism of Action

- Relies on pre-existing antibody response
- Complement mediated destruction of vaccine cells, immune-activation and cross-presentation of tumor antigens
- Tumor specific CD8+ cytotoxic T cells are generated to recognize patient's own tumor
- Tumor specific immune response recognizing shared tumor antigens is generated post vaccination

PANCREATIC CANCER HISTORIC PERSPECTIVE

- 4th leading cause of cancer death in U.S. (6)
- All stages, 5 year survival < 5% (7)
- Stage IIB, resected, 5 year survival <8% (6)
- Resection rate 20-25% U.S. (6)
- Post resection standard of care
  - Chemotherapy +/- Radiotherapy
  - Gemcitabine +/- 5 FU Concurrent Radiotherapy

Annual Incidence			
TOTAL	US	EUROPE	JAPAN
117,000	43,000	45,000	29,000

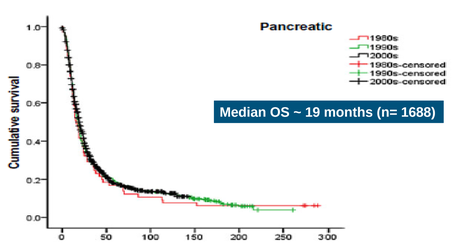
RESECTED PANCREATIC CANCER: ASSESSMENT OF RISKS FACTORS: *NLG0205 and RTOG9704[9]*

Risk Factor		Hazard Ratio	*p* value	
Tumor Size				
RTOG9704	< 3 cm	1.21	0.12	High Risk Prognostic Indicators
	≥ 3 cm			
NLG0205	< 2.5 cm	1.59	0.21	
	≥ 2.5 cm			
Nodal Status (RTOG9704)				
	N0	1.53	0.001	
	N+			
Resection Margins (RTOG9704)				
	R0	1.05	0.74	
	R1			

High Risk Prognostic Indicators : Positive Nodes (N+) and/or Tumor Size ≥ 2.5 m

*Resection Margin is **NOT** an independent prognostic indicator in these data set*

RESECTED PANCREATIC CANER: SURVIVAL ANALYSIS BY DECADE



Objective:
This study was carried out to determine relative survival rates and trends in outcomes in patients who underwent resection of periampullary adenocarcinomas (PACs) with curative intent at a single institution over the last three decades

Conclusion:
The overall long term outcomes have not improved significantly (8)

NLG0205 PHASE 2 ALGENPANTUCEL-L: STUDY OVERVIEW

- Design: open-label, 71 patient (n=69 evaluable) multicenter phase 2 study evaluating algenpantucel-L plus standard of care (SOC) gemcitabine with 5-FU-XRT for resected pancreatic cancer
- Endpoints: DFS at 1 year, OS, correlative immunologic analysis
- Eligibility: Post-resection patients with no evidence of residual disease, SOC (gemcitabine+5 FU+Concurrent XRT) + algenpantucel-L
- Treatment Schedule: SOC (gemcitabine with 5-FU-XRT) plus algenpantucel-L, Q2weeks X 6 months
  - Two dose cohorts: low dose (100 million cells) and high dose (300 million cells)
- Correlative immunologic analysis: Patients with samples before and after immunization were evaluated for the induction of anti-αGal Ab, anti-CEA Ab, anti-mesothelin Ab and anti-CALR Ab by ELISA

NLG0205 PHASE 2 : PATIENT CHARACTERISTICS

Characteristics		RTOG-9704	NLG0205
Age (Median)		61	62
Gender (Male)		53%	52%
Tumor Location	Head	85%	84%
	Body/Tail	15%	16%
CA19-9 (≥180)		9%	18%
Tumor Grade (Poor/Undifferentiated)		30%	81%
Nodal Status	(N+)	68%	81%
Tumor Size (Median)	≥3.0 cm	59 %	66%
High Risk	***N+ and/ or ≥ 2.5 cm***	***NA***	***96%***
Low Risk	**N0 and < 2.5 cm and R0/R1**	***NA***	***4%***

NLG0205 PHASE 2: RESULTS

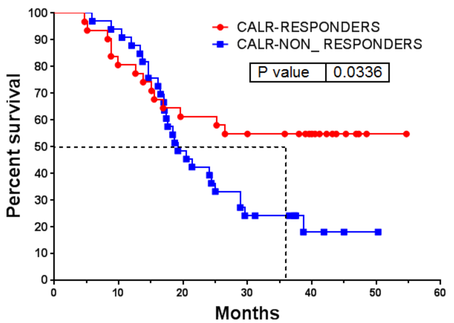
- Multicenter(16), open label, 2 arm study (n=69 evaluable)
- Adverse Events: grade 1or 2 skin reactions at injection sites (51%)
  - Grade 3 events possibly attributable to vaccine:
    - Lymphopenia (6%), skin reaction/pain (3%) and leukopenia/neutropenia (3%)
  - No grade 4 drug related adverse events reported
- Primary endpoint met: 1 year DFS 62%
  - DFS: High dose (81%) superior to low dose (52%) p= 0.02
- Secondary endpoints: 1 year Overall Survival (OS)
  - OS: High dose (96 %) superior to low dose (79%) p= 0.049
- Previous correlative immunologic studies: increased in anti-αGal Ab, anti-mesothelin and/or anti-CEA Ab correlated with improved overall survival
  - Multi-parameter analysis indicated that a combination of more than one antibody response correlates with improved survival

NLG0205 PHASE 2 : CORRELATIVE IMMUNOLOGIC STUDIES

Assessed Parameter: Anti-Calreticulin Ab response after immunization

Anti-CALR Ab	Increase Ab	No Increase	Total
Counts	31	33	64
OS (months)	>36	19.2	P< 0.04 (log rank test)
Survival Rate at 36 months	55%	21%	P <0.01 (Fisher's exact test)

Figure 2: Anti-CALR response and Correlation with Survival



- Anti-CALR Ab increase ≥20% vs. baseline considered significant
- 64 evaluable patients were tested with samples before and after immunization
- Increased in anti-CALR correlates with improved survival

NLG0205 PHASE 2 ALGENPANTUCEL-L: CONCLUSIONS

- Elevation of anti-Calreticulin Ab
  - Correlates with improved survival
  - Suggests immune activation by algenpantucel-L
  - Potential predictive value for subsequent treatment decisions
- Nodal status and tumor size are important prognostic indicators
  - Resection margin is not an independent prognosis indicator
- NLG0205 patient's characteristics: 96% high risk patients represented by equal to or larger than 2.5 cm tumor or N+

IMPRESS PHASE 3 REGISTRATION TRIAL, NLG0405 (n = 722)

- Initiated, May 2010 under SPA with Fast Track and Orphan Drug designation by the FDA
- Open label, 2 arm, 1:1 randomized study enrolling resected pancreatic cancer patients
- SOC +/- algenpantucel-L (SOC = gemcitabine +/- radiation)
- Algenpantucel-L: 300 million cells Q2wks X 6 mo → Q1m X 6 mo
- Overall Survival is the primary endpoint
- Stratified for Nodal Status, Radiotherapy and CA 19-9
- Accrual Status and Endpoints
  - Completed enrollment September 2013 (722 patients)
  - Early interim analysis (222 events) completed: No unanticipated safety events; DSMC recommendation continue without modification
  - Second interim analysis at 333 events; Final analysis at 444 events (if required)
  - Designed to detect ≈20% difference in overall survival at final analysis
- ClinicalTrials.gov Identifier: NCT00569387

IMPRESS NLG0405 PHASE 3 : PATIENT CHARACTERISTICS

Characteristics		RTOG-9704	NLG0405
Age (Median)		61	65
Gender (Male)		53%	52%
Tumor Location	Head	85%	80%
	Body/Tail	15%	20%
CA19-9 (≥180)		9%	9%
Tumor Grade (Poor/Undifferentiated)		30%	35%
Nodal Status	(N+)	68%	70%
Tumor Size (Median)	≥3.0 cm	59 %	55%
High Risk	***N+ and/ or ≥ 2.5 cm***	***NA***	***92%***
Low Risk	**N0 and < 2.5 cm and R0/R1**	***NA***	***8%***

IMPRESS Patient Characteristics are consistent with other large US based trials with 92% high risk and 8% low risk patients.

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