Sipuleucel-T immunotherapy for castrate-resistant prostate cancer: Elucidating mechanism of action

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Aim

Find evidence of treatment response, immune activation and mechanism of action for patients with metastatic castrate-resistant prostate cancer undergoing treatment with sipuleucel-T.

Background

Immunotherapy and prostate cancer

- 3 E's of immunoediting[1]: elimination, equilibrium, escape
- Emerging interest in promoting immune-mediated killing of cancer cells (cytokines, checkpoint inhibition, vaccines)
- Potential advantages in prostate cancer: slow growth, tissue-specific antigens and non-essential target, reliable serum marker of tumor burden
- B7-H3 is an immune checkpoint molecule widely expressed by prostate cancer cells associated with increased tumor aggression[2]

Sipuleucel-T

- Autologous cell based immunotherapy (FDA approved 2010) used in the treatment of asymptomatic or minimally symptomatic metastatic castrate-resistant prostate cancer (mCRPC)
- Manufactured by *ex vivo* culture of patient's peripheral blood mononuclear cells with PA2024 fusion protein: prostatic acid phosphatase (PAP) + GM-CSF
- Median OS benefit = 4.1 months (IMPACT)[3]
- Improved OS correlated with low PSA burden[4], higher peripheral Ab titers[5], transient eosinophilia[6], "antigen spread"[7]

Current gaps

- **How do we know it's working?** Despite OS benefit, there is no change in traditional markers of response to therapy (time to progression, PSA response).
- **How does it work?** Developed to activate Ag-specific T cell response by promoting differentiation of dendritic cells but no definitive evidence of this.

1) Dunn et al. (2004) *Annu Rev Immunol* 22, 329.
2) Zang et al (2007) *Proc Natl Acad Sci* 104, 19458
3) Kantoff et al. (2010) *N Engl J Med* 363, 411.
4) Schellhammer et al. (2013) *Urology* 81, 1297.
5) Sheikh et al. (2013) *Cancer Immunol Imm* 62, 137.
6) McNeel et al. (2014) *Cancer Immunol Res* 2, 1998
7) GuhaThakurta et al. (2015) *Clin Cancer Res* 21, 361

Methods

Clinical Trial NCT02036918 (see Trial Design)

Sample processing

- Serum samples were taken at baseline, prior to each sipuleucel-T leukapheresis session and after the last infusion
- Serum CBC with differential, PAP and PSA levels were obtained as standard lab evaluations

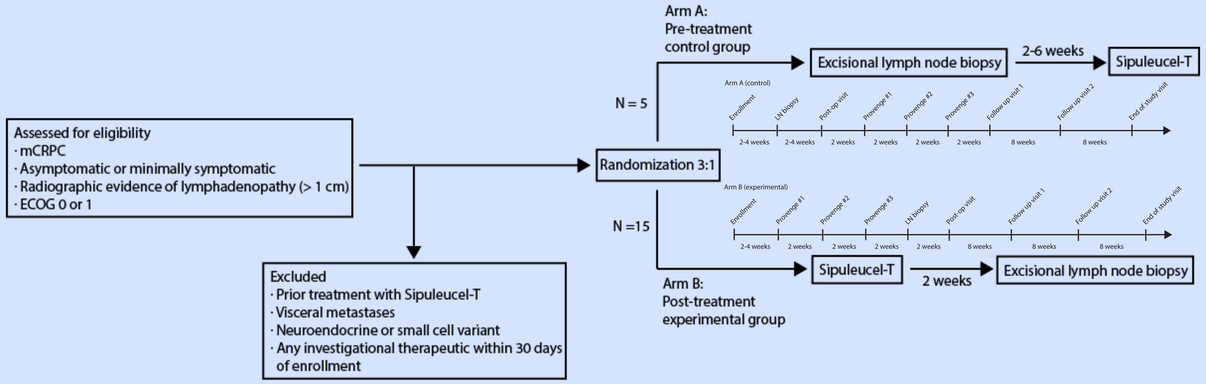
Immune assays

- Serum anti-PA2024, anti-PAP and anti-tetanus levels were measured using ELISA (performed by Dendreon Pharmaceuticals Inc.)
- Serum cytokine levels were measured using a 30-plex human cytokine panel developed for the Luminex Immunobead assay system
- Serum soluble B7-H3 (sB7-H3) levels were tested in triplicate using ELISA calibrated against highly purified NS0-expressed recombinant human B7-H3. Pre- and post- levels were compared using a paired t-test.

Treatment summary

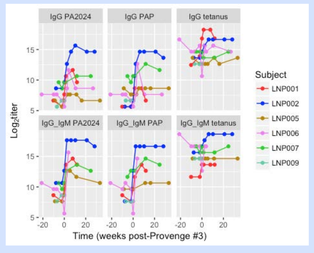
Subject	Arm	Surgery type / Location	Pre-PSADT (mo)	Post-PSADT (mo)
LNP001	B	Open / Inguinal + Pelvic	15.9	1.9
LNP002	B	Lap / Retroperitoneal	2.3	2.3
LNP004	B	Lap / Retroperitoneal	3.3	3.3
LNP005	B	Open / Pelvic	2.2	n.a.
LNP006	A	Open / Retroperitoneal + Pelvic	0.5	4.2
LNP007	B	Open / Pelvic	1.7	1.7
LNP009	B	Lap / Retroperitoneal	4.3	4.3

Trial design

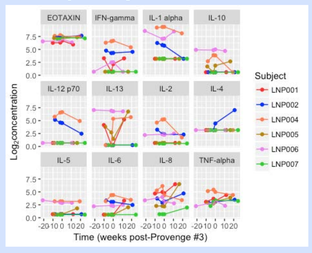


Peripheral immune response - T_H2 phenotype?

Ag-specific response



Multiplex cytokine detection



LNP002

Highest Ag-specific titers
Transient eosinophilia present

Known correlates with OS benefit

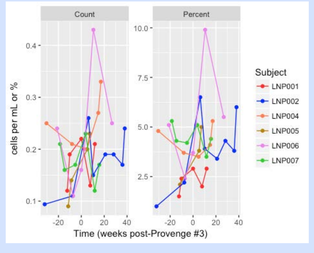
↓ IL-12 (T_H1 cytokine)
↑ IL-4 (T_H2 cytokine)

T_H2 phenotype

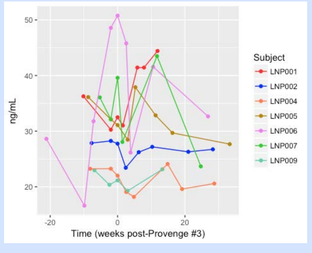
↓ IL-6
↓ IL-1alpha
↓ sB7-H3

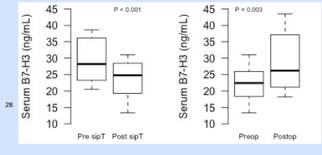
Decreased markers of disease aggression

Serum eosinophils



Serum sB7-H3





Reduced production of sB7-H3 with treatment

Post-op inflammation increases sB7-H3

Mechanistic framework for immunotherapy

$$\frac{d[T]}{dt} = k_{grow}[T] - k_1[T][E] + k_{-1}[TE]$$

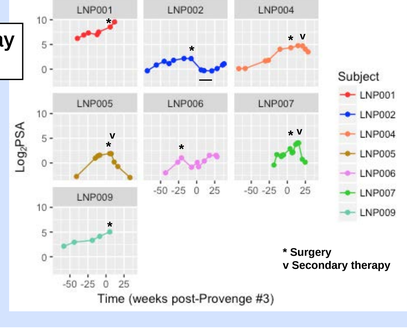
$$\frac{d[E]}{dt} = -k_1[T][E] + k_{-1}[TE] - k_{decay}[T] + k_{elim}[TE]$$

$$\frac{d[TE]}{dt} = k_1[T][E] - k_{-1}[TE] - k_{elim}[TE]$$

[T] = tumor burden
[E] = immune effector
k_{elim} = killing rate per immune effector
k_{-1} / k_1 = apparent effector-tumor binding affinity
k_{decay} = decay rate of immune effect

- Based on equations developed previously[8,9]
- Assumes immune killing is rate limiting

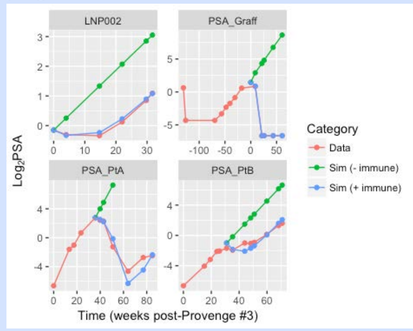
LNP002: Unique 15 week delay in growth following surgery



8) dePillis et al. (2014) *J Pharmacokinet Pharmacodyn 41*, 461. 9) Kuznetsov et al. (1994) *Bull Math Biol* 56, 295

Connecting the dots

Quantifying the immune effect

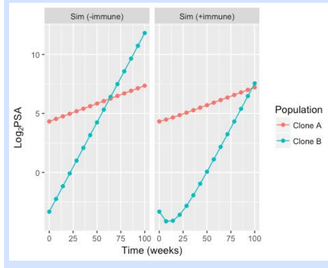


Subject	k_{grow} ng/mL/wk	k_{-1}/k_1 ng/mL	[Effector] k_{elim} ng/mL/wk	$t_{1/2}$ (decay) weeks
LNP002	0.07	1	0.22	5
Graff*	0.08	0.1	0.28	5
Pt A**	0.21	1	1.93	5
Pt B**	0.13	1	0.46	5

* Data adapted from published case report of exceptional responder[11]
** Exceptional responders from internal retrospective review

Note: Ab titer half life = 4-16 weeks[10]

How can we explain overall survival benefit with Sip-T?



Assumptions:
- Branching process model of cancer; this is consistent with evolution towards faster PSADT
- Divergent clones have accumulated more mutations and are more immunogenic. Role of "antigen spread."[7]

Simulated data shows potential basis for delayed and "hidden" survival benefit of immunotherapy

10) Burch et al. (2004) *Prostate* 60, 197.
11) Graff et al. (2013) *Urology* 81, 381.

Conclusions

Sipuleucel-T may act via T_H2-dominant (humoral) pathway

- Contrary to purported cytotoxic mechanism of action (T_H1-dominant)
- Unique responder (LNP002) with increased IL-4, decreased IL-12
- Consistent with prior studies showing correlation between OS and high Ab titers, transient eosinophilia.[5,6]
- Predicted k_{decay} consistent with serum half-life of Ab titers

Hypothesis-generating mechanistic framework for immune action

- Estimated effector killing can account for OS benefit noted in several clinical trials by targeting smaller populations of aggressive, immunogenic tumor cells that may not correspond to the dominant clone(s) driving PSA changes
- Consistent with decreased markers of aggressive disease in LNP002

Future work

- Completion of ongoing clinical trial, including measuring immune response of excised lymph nodes (ELISPOT, TCR sequencing)
- Apply better quantitative methods and equations to assess and compare response to secondary therapies that decrease tumor burden in combination with Provenge +/- other immunotherapies

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Duke Cancer Institute