Tim Illidge
Radiotherapy and immunotherapy combinations - unlocking the potential

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Improving outcome for cancer patients with Radiotherapy and immunotherapy combinations

- Developing technologies in RT eg Stereotactic Body Radiotherapy (SBRT), SABR, SRS precise delivery of **highly conformal and image-guided hypofractionated** EBRT: single or hypofractionated EBRT $\geq$ BED to conventional fractionation

- Novel immunomodulatory agents: combination with RT - unique opportunity, lack of systemic toxicity of localised treatment – **immunogenic hub to break tumour tolerance**

- **RT with immunomodulatory agents to enhance systemic immunity**
  - Local disease: Improving local control and decrease development of systemic metastases
  - Metastatic disease: using RT to enhance systemic immunity,

- In CTCL lymphoma – exquisite radiosensitivity local RT: large amounts of immunogenic cell death, TSEBT
> 350 immune-based clinical trials currently open in oncology (NCI database search term ‘immunotherapy and cancer’)

Immunotherapy earns its spot in the ranks of cancer therapy

Feb. 2012

J. Exp. Med. Vol. 209 No. 2 201-209

Drew Pardoll and Charles Drake

Cancer immunotherapy comes of age

Ira Mellman¹, George Coukos² & Glenn Dranoff³
Developing effective combinations of radiotherapy and immunotherapy in cancer

Immunomodulatory agents to enhance T cell response
Some of the key research questions in combining RT with immunotherapy

• Is radiation (RT)-induced cancer cell death immunogenic and does it stimulate a host immune response?

• Can combination of RT with immuno-modulatory agents enhance anti-tumour immune responses?

• How are immunogenicity and therapeutic outcome influenced by:
  > RT dose, fractionation & scheduling
  > Intrinsic cell death associated factors including release of damage-associated molecular patterns (DAMP)
  > Extrinsic micro-environmental factors
Key research questions in combining RT with immunotherapy

• Can cancer cell death be immunogenic and stimulate a host immune response?

• Can combination of RT with immuno-modulatory agents enhance anti-tumour immune responses?

• How is immunogenicity and therapeutic outcome influenced by:
  > RT dose, fractionation & scheduling
  > Intrinsic cell death associated factors including release of damage-associated molecular patterns (DAMP)
  > Extrinsic micro-environmental factors
Production of reverse Caspase-3 “death switch” cells.
Induction of death switch in vitro shows apoptotic cell death
Death switch cells release endogenous danger signals

24 Hours after treatment

WT

Death switch

Supernatant of cells, 24 Hours

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Transmission electronic microscopy
Induction of death switch *in vivo* results in tumour regression
Induction of death switch *in vivo* results in infiltration of immune effector cells

24 Hours after Doxycycline

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Sustained tumour eradication after induced caspase-3 activation and synchronous tumour apoptosis requires an intact host immune response


Doxycycline administrated for 7 Days

Re-challenge with B16 OVA shows a trend in delayed tumour growth
Summary caspase-3 “Death switch” models

• Doxycycline-dependent caspase 3 “death switch” model encodes reverse caspase 3 molecule which induces cell death within 24hrs displaying classical features of apoptosis

• Induces apoptotic cell death both in vitro and in vivo

• Long tumour clearance requires an intact immune system

• Provides opportunities to develop a range of tumour models to study immune response to “pure” apoptosis in vivo compared with other apoptosis inducing agents such as radiation therapy and chemotherapy
The problem: Tumours and systemic chemotherapy can induce profound local immunosuppression.

- Irradiated tumour cells
- Release of calreticulin and endogenous danger signals eg. HMGB1, HSP, uric acid, ATP
- Absence of T cell activation
- Can induce poor activation of DC leading to a ‘tolerogenic’ phenotype leading to immunological anergy
- Complex milieu of tumour / stromal derived immunosuppressive cytokines i.e. IL-10, TGFβ, PGE2 and immune cells i.e. Treg, Mφ2, MDSC
- Absence of NK cell activation
- Absence of T cell activation
T-cell immune check-points in the tumour microenvironment

**Antigen Presenting Cell**

- **B7-H3 / CD276**
- **B7-H4 / B7X / B7S1 / VTCN1**
- **B7DC / PDL2 / PDCD1LG2 / CD273**
- **B7H1 / PDL1 / PDCD1LG1 / CD274**
- **B7.1 / CD80**
- **B7.2 / CD86**

**T Cell**

- **B7-H3 / CD276**
- **B7-H4 / B7X / B7S1 / VTCN1**
- **B7DC / PDL2 / PDCD1LG2 / CD273**
- **B7H1 / PDL1 / PDCD1LG1 / CD274**
- **B7.1 / CD80**
- **B7.2 / CD86**

**Inhibitory receptors**

- **PD-1 / PDCD1 / CD279**
- **CTLA-4 / CD152**
- **CD28**

**Activatory receptors**

- **LAG-3 / CD223**
- **GITR / AITR / TNFRSF18**
- **OX40 / ACT-135 / TNFRSF4 / CD134**
- **CD137 / 4-1BB / ILA / TNFRSF9**
- **ICOS / CD278 / AILIM / CRP-1**

**MHC**

- **GITRL / AITRL / TNFSF18**
- **OX40L / gp34 / CD252**
- **CD137L / 4-1BBL / TNFSF9**
- **ICOSL / B7H2 / GL50 / B7RP1 / CD275**
Key research questions in combining RT with immunotherapy

• Is radiation (RT)-induced cancer cell death immunogenic and does it stimulate a host immune response (and lead to abscopal responses and systemic immunity)?

• Can combination of RT with immuno-modulatory agents enhance anti-tumour immune responses and overcome immunosuppression?

• How is immunogenicity and therapeutic outcome influenced by:
  > RT dose, fractionation & scheduling
  > Intrinsic cell death associated factors including release of damage-associated molecular patterns (DAMP)
  > Extrinsic micro-environmental factors
Mouse tumour modelling: RT and Immunotherapy Combinations

Radiotherapy

Immunotherapy

Generate durable anti-tumour immune response to Tumour
Targeting immuno-regulatory checkpoints to overcome immune suppression

- Stimulatory receptors
  - TLR7
  - OX40-L
  - CD40

- APC
  - Peptide-MHC/TCR ligation
  - OX40
  - CD40-L

- Inhibitory receptors
  - CD73
  - B7
  - PD-L1
  - CTLA-4
  - PD-1

mAb and small molecule agonists

T-cell

Anti-tumour T-cell response
Anti-CD40 monoclonal antibody therapy in combination with irradiation results in a CD8 T-cell–dependent immunity to B-cell lymphoma Blood. 2003; 15;102(4):1449-57
Jamie Honeychurch, Martin J. Glennie, Peter W. M. Johnson, and Tim M. Illidge
RT and anti-CD40 induce long term anti-tumour immunity

Splenocytes (including tumour specific CD8 T-cells)

Anti-CD40

Days post-tumour inoculation

% Survival

PBS
Control
Splenocytes

% survival

Day post tumour inoculation

Naive
Treated
• TLR’s class of proteins play a key role in the innate immune system
• 32 open clinical trials of TLRs in cancer
• Selective TLR7/8 agonist Imiquimod approved for topical treatment of BCC (topical)
Systemic delivery of a TLR7 agonist in combination with radiation primes durable antitumor immune responses in mouse models of lymphoma

Simon J. Dovedi,¹ Monique H. M. Melis,¹ Robert W. Wilkinson,² Amy L. Adlard,³ Ian J. Stratford,³ *Jamie Honeychurch,¹ and *Timothy M. Illidge¹

Plenary paper. Blood, 10 January 2013 - VOLUME 121, NUMBER 2
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**EG7 model of T cell Lymphoma**

- Control
- 10Gy + 3mg/kg R848 q1w
- 10Gy + 3mg/kg R848 q1w + αCD8 mAb

**EL4 model of T cell Lymphoma**

- Control
- 5x2Gy RT
- 3mg/kg R848 q1w
- 3mg/kg R848 q1w + 5x2Gy RT

**A20 model of B cell Lymphoma**

- NT
- 5x2Gy RT
- 3 mg/kg R848 q1w
- 5x2Gy RT + 3 mg/kg R848 q1w
In situ vaccination with TLR9 agonist induces lymphoma regression; Phase I/II study Brody et al JCO; 2010: 28

Intratumoral vaccination induces objective clinical responses [extended]. (A) Complete response in patient treated site: occipital; visualized site: bilateral axillae. (B) Partial response in patient’ treated site: suprasternal cutaneous; visualized site: supra-orbital cutaneous
• In situ vaccination with TLR9 agonist induces lymphoma regression; Phase I/II study Brody et al JCO; 2010: 28

Intratumoral vaccination induces objective clinical responses. (A) Patients received 2 Gy × 2 radiation combined with intratumoral injection of PF-3512676 to a single disease site and disease was measured at up to six distant sites. (B) Waterfall plot showing percent change in the cross-product sum at the time of best response (indicated above ordinate) versus pretreatment.
Targeting immuno-regulatory checkpoints: CTLA4

**Tremelimumab** (CP-675,206)  
*MedImmune*

~17 trials Phase I-III in melanoma, pancreatic, colo-rectal, prostate, bladder, renal, NSCLC

**Ipilimumab** (MDX-010/ MDX-101)  
*Bristol-Myers Squibb*

~16 trials in melanoma, prostate, pancreatic
Targeting immuno-regulatory checkpoints to overcome immune suppression: PD1/PD-L1 signalling axis

Programmed cell death ligand 1 (PD-L1) is an immunomodulatory molecule expressed by APC's and select tumours that engages receptors on T cells to inhibit T-cell immunity.

Therapeutic blockade of the T cell negative regulator (PD-L1 / B7-H1) can enhance T cell effector function when PD-L1 is expressed in tumours. Topalian et al; Brahmer et al NEJM 2012; > 38 clinical trials in progress.)

- PD-L1 can be expressed by Reed-Sternberg cells comprising classical Hodgkin lymphoma (CHL) and by malignant B cells comprising EBV-positive PTLD.

- Aim to determine whether expression of PD-L1 represents a general strategy of immune evasion among aggressive B-cell lymphomas and virus- and immunodeficiency-associated tumors.

- FFPE tissue biopsies, examined 237 primary tumors for expression of PD-L1.

- PD-L1 protein expression found in the majority of nodular sclerosis and mixed cellularity CHL, primary mediastinal large B-cell lymphoma, T-cell/histiocyte-rich B-cell lymphoma, EBV-positive and -negative PTLD, and EBV-associated diffuse large B-cell lymphoma (DLBCL), plasmablastic lymphoma, extranodal NK/T-cell lymphoma, nasopharyngeal carcinoma, and HHV8-associated primary effusion lymphoma. Within these tumors, PD-L1 was highly expressed by malignant cells and tumor-infiltrating macrophages.

- In contrast, neither the malignant nor the nonmalignant cells comprising nodular lymphocyte-predominant Hodgkin lymphoma, DLBCL-not otherwise specified, Burkitt lymphoma, and HHV8-associated Kaposi sarcoma expressed detectable PD-L1.

- Certain aggressive B-cell lymphomas and virus- and immunodeficiency-associated malignancies associated with an ineffective T-cell immune response express PD-L1 on tumor cells and infiltrating macrophages.

- Results identify a group of neoplasms that should be considered for PD-1/PD-L1-directed therapies, and validate methods to detect PD-L1 in FFPE tissue biopsies.
Evaluated the clinical impact of sPD-L1 level measured at the time of diagnosis for newly diagnosed DLBCL in plasma of 288 patients in a multicenter, randomized phase III trial that compared R-high-dose chemotherapy to R-CHOP.

Median follow-up was 41.4 months. A cut-off of 1.52 ng/ml of PD-L1 level was determined and related to overall survival (OS).

Patients with elevated sPD-L1 experienced a poorer prognosis with a three-year OS of 76% versus 89% (P<0.001).

The intention-to-treat analysis showed that elevated sPD-L1 was associated with a poorer prognosis for patients randomized within the R-CHOP arm (P<0.001).

Plasma PD-L1 protein is a potent predicting biomarker in DLBCL and may indicate usefulness of alternative therapeutic strategies using PD1 axis inhibitors.

• Endogenous or iatrogenic antitumour immune responses can improve the course of follicular lymphoma, but might be diminished by immune checkpoints in the tumour microenvironment – including PD1, a co-inhibitory receptor that impairs T-cell function and is highly expressed on intratumoral T cells.

• Phase 2 trial of the activity of pidilizumab, (humanised anti-PD1 mAb), with rituximab in patients with relapsed follicular lymphoma.

• Adult (≥18 years) patients with rituximab-sensitive follicular lymphoma relapsing after one to four previous therapies were eligible.

• Pidilizumab administered at 3 mg/kg intravenously q4 weeks for 4 infusions, plus eight optional infusions every 4 weeks for patients with stable disease or better. Starting 17 days after the first infusion of pidilizumab, rituximab was given at 375 mg/m\textsuperscript{2} intravenously weekly for 4 weeks.

• Primary endpoint was the proportion of patients who achieved an objective response (complete response plus partial response according to Revised Response Criteria for Malignant Lymphoma).

• Of 29 patients evaluable for activity, 19 (66%) achieved an objective response: complete responses were noted in 15 (52%) patients and partial responses in four (14%).
Safety and activity of PD1 blockade by pidilizumab in combination with rituximab in patients with relapsed follicular lymphoma: a single group, open-label, phase 2 trial.

- 32 patients enrolled between; Median follow-up was 15.4 months (IQR 10.1-21.0).

- Combination of pidilizumab and rituximab was well tolerated, with no autoimmune or treatment-related adverse events of grade 3 or 4.

- The most common adverse events of grade 1 were anaemia (14 patients) and fatigue (13 patients), and the most common adverse event of grade 2 was respiratory infection (five patients).

- Of the 29 patients evaluable for activity, 19 (66%) achieved an objective response: complete responses were noted in 15 (52%) patients and partial responses in four (14%).

- Combination of pidilizumab plus rituximab is active in patients with relapsed follicular lymphoma and requires further study.
Irradiation and anti-PD-L1 treatment synergistically promote anti-tumor immunity in mice.
Deng L, Liang H, Burnette B Beckett M, Darga T, Weichselbaum RR, Fu YX
J Clin Invest. 2014 Feb 3;124(2):687-95

- PD-L1 upregulated in the tumour microenvironment after RT.
- Administration of anti-PD-L1 enhanced the efficacy of RT through a cytotoxic T cell-dependent mechanism.
- RT and anti-PD-L1 synergistically reduced the local accumulation of tumour-infiltrating MDSCs, which suppress T cells and alter the tumour immune microenvironment.
- Activation of cytotoxic T cells with combination therapy mediated the reduction of MDSCs in tumours through the cytotoxic actions of TNF.
- Establishes a basis for the rational design of combination therapy with immune modulators and radiotherapy
Blockade of PD1 or PD-L1 leads to an improvement in survival and to the induction of protective immunological memory.

- **NT**
- **αPD-1 mAb 10 mg/kg**
- **5x2Gy RT**
- **5x2Gy RT + αPD-1 mAb 10 mg/kg**

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**Tumour rechallenge**

- **NT**
- **αB7-H1 mAb 10 mg/kg**
- **5x2Gy RT**
- **5x2Gy RT + αB7-H1 mAb 10mg/kg**
Efficacy of RT/\(\alpha\)PD-L1 combination is CD8\(^+\) T cell dependent

- NT
- 5x2Gy RT + \(\alpha\)B7-H1 10mg/kg 3qw
- 5x2Gy RT + \(\alpha\)B7-H1 + \(\alpha\)CD8 mAb
- 5x2Gy RT + \(\alpha\)B7-H1 + \(\alpha\)CD4 mAb
- 5x2Gy RT + \(\alpha\)B7-H1 + \(\alpha\)AGM1 mAb

Percent survival

Time after tumour innoculation (days)
Summary

- RT induces immunogenic tumour cell death and understanding more about the effects of RT dose and fractionation on DAMP release and extrinsic micro-environmental factors is important to developing the most effective regimens.

- The efficacy of RT may be enhanced through combination with immunomodulatory agents and understanding more about RT dose, fractionation and scheduling is key to unlocking the potential of such combination approaches.

- Translation of these novel therapeutic approaches into the clinic are warranted and key to improving outcome for patients receiving RT.
Acknowledgements

Targeted Therapy Group
- Simon Dovedi
- Jamie Honeychurch
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- Grace Lipowska-Bhalla
- Clara Chan
- Conor McKenna
- Benjamin Sanderson
Potential clinical studies

- Phase I/II in CTCL using TSEBT + TLR 7 / 9 or anti-PD1 / PDL1
  - TLR-7 (DSP, Japan; MonTa Biosciences Liposomal TLR-7)
- Phase I / II in CTCL with external beam RT and TLR-7 or anti-PD1 or anti-CTLA4
- Translational study looking at PDL1 expression post –RT
  - Is PDL1 adaptive resistance mechanism to RT