Title: Inducing immunogenicity in rectal cancer: A Phase I/II trial of aspirin +/- PD1 inhibitor in combination with neoadjuvant chemoradiation therapy for locally advanced rectal cancer: The INTIMACCI study (Inducing Tumour IMMunogenicity with Aspirin, chemoradiation and Checkpoint Inhibition)

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Preparation: Sara Wahlroos

Introduction:

Pathological complete response rate (pCR) to neoadjuvant chemoradiation (CRT) for patients with locally advanced rectal cancer is associated with improved disease-free and overall survival. This makes the neoadjuvant setting an ideal setting to test the efficacy of novel therapies, as pCR is an early and definitive endpoint.

Immunotherapy, in particular PD1/PDL1 inhibitors, have shown significant promise in multiple tumour types, however, in colorectal cancer (CRC), activity has been limited to those with microsatellite unstable cancers. Therefore, in CRC, the challenge is to induce tumour immunogenicity in order to harness benefit from these agents. Data support the role of both radiotherapy and aspirin in generating an immune competent microenvironment that may improve efficacy from PD1/PDL1 blockade. Hence, neoadjuvant therapy for rectal cancer (RC) appears to be an ideal setting to add these agents to improve outcomes. This study aims to test the combination of aspirin and a PD-1 inhibitor, together with CRT, with the primary endpoint of pCR and exploratory translational secondary endpoints.

Rationale:

1. Only microsatellite unstable CRC has shown response to PD-1 inhibitors. CRC with high density tumour infiltrating lymphocyte (TIL) infiltrate is associated with improved prognosis. Therefore inducing an immune/inflammatory response is likely to improve outcome.
2. RT is a potent inducer of local and systemic (abscopal) immune response. Neoadjuvant radiotherapy for rectal cancer is associated with increased inflammatory cell infiltrate in resected specimens.
3. Aspirin modulates the immune microenvironment and increase the likelihood of benefit from PD-1 inhibitors. Pre-clinical models have shown that aspirin downregulates COX and consequently decreases production of Prostaglandin E2. By doing this, IL-6 production is decreased, while INF-γ is increased, which has been associated with increased response rates to PD-1 inhibitors.
4. Aspirin is independently synergistic with radiation therapy.
5. An increase in the tumour inflammatory response, in particular an immune competent response, is associated with increased benefit from PD-1 inhibitors.
6. Therefore there is a strong rationale for combining standard chemoRT with aspirin and PD1 inhibitors to observe if there is a provoked immune response and subsequent improvement in pCR.
Benefits of neoadjuvant model:

- Use of **tumor as an in-situ vaccine** (large quantities of tumor presented to the immune system in an immunogenic fashion)
- Accessibility of tumor for serial biopsy and analysis of post-operative resection specimen
- Excellent **surrogate endpoint of pathological complete response** (pCR) which correlates with long term survival.

Background:

**Radiotherapy and immunogenicity**

The immunological effects of radiotherapy-induced tumour cell death includes:

1. **Enhancement of MHC class I surface expression**, calreticulin expression, and activation of the damage-associated molecular pattern (DAMP) – associated high motility group box 1 (HMGB1). (Figure 1, Sharabi et al. 2015).\(^1\) Upregulation of MHC class I molecules causes increased activation of CD8 T cells, which form the major cell-mediated cytotoxic arm of the adaptive immune system. Release of HMGB1 act as a proinflammatory mediator and activates dendritic cells, which then primes the immune system to activate and proliferate tumour-specific CD8 Tcells.\(^1,2\)

![Figure 1](image.png)

**Fig1.** Radiation induced changes to the tumour cell immunophenotype. Radiation-induced DNA and membrane damage, and cytoplasmic reactive oxygen species (ROS) activate many transcription factors and signaling pathways that modulate the immunophenotype and immunogenicity of tumour cells.\(^1\)

2. **Cytokine release.** The systemic or abscopal effects of localized radiation may be driven by the induction of a proinflammatory cytokine storm, with release of TNF-\(\alpha\), INF-\(\gamma\), IL-1b, IL-12 by non malignant cells which evokes a systemic immune response that may control the growth of metastases outside the field of radiation.\(^3\)

3. **Increase in chemokines and expression of adhesion molecules**, such as CXCL9, CXCL10 - induced by IFN-\(\gamma\), and CXCL 16, promote chemotaxis and migration of CD4 and C8 T cells into the irradiated tumour. Tumours with high levels of CXCL16 expression has been correlated with increased numbers of TILs and better prognosis.\(^1,3\)
4. Increase tumour sensitivity to check point inhibitors. Radiation modulates the expression of immune checkpoint ligands, including PD-1L, on the surface of tumour cells and on the immune cells in the tumour microenvironment.\(^1\) The upregulation of PD-1L was shown in one study to be driven by INF-\(\gamma\) derived from CD 8 \(T\) cells. The group also showed that concurrent therapy rather than sequential was needed to improve survival in pre-clinical models.\(^4\) Antibody binding alone is not predominantly cytotoxic, however, serves as a marker and platform for antibody-dependent cellular cytotoxicity and non-cell-mediated cytolysis through the fixation of complement.\(^1\) (Figure 2, Sharabi et al. 2015) It has been shown that radiotherapy increases MHC class 1 expression in a dose-dependent manner, which is critical as its upregulation renders the tumour cells detectable by CD8 \(T\) cells and immune killing.\(^5,6\) However, the radiation dose and fractionation needed to optimize the interaction with checkpoint blockade immunotherapy is still unknown.\(^3\)

![Fig 2. Radiation combined with checkpoint blockade increases tumour cell susceptibility to immune-mediated cell death. A) Anti-PD-L1 antibody alone is not predominantly cytotoxic. B) RTx in combination with anti-PD-L1 upregulates MHC and FAS on tumour cells, increasing susceptibility to \(T\) cell mediated cytotoxicity. TCR = \(T\) cell receptor](image)

Aspirin and immunogenicity

Both the anti-inflammatory and antiplatelet actions have been linked to the anticancer activity of aspirin. Low-dose aspirin delays inflammation-driven tumour progression \textit{in vivo}, shifting the tumour inflammatory profile towards classic anti-cancer immune pathways.\(^7\) The antiplatelet activity of aspirin may prevent tumour thrombus formation, limiting metastatic potential.\(^8\) Other potential mechanisms include inhibition of NFkB, Wnt, TGF, insulin and mTOR signalling,\(^9,10\) induction of apoptosis by activation of p38 kinase and catabolism of polyamines,\(^12\) normalization of EGFR expression,\(^13\) downregulation of c-Myc and Sp transcription factors,\(^14\) TP53 activation\(^15\) and hTERT\(^16\) inhibition. Many studies have supported the finding that regular intake of aspirin can affect colorectal carcinogenesis through COX inhibition and reduced synthesis of Prostaglandin (PG) E2. Cyclooxygenase (COX)1 and COX-2 enzymes are critical for the production of PGE2, and are often overexpressed in CRC cancer.\(^7,17\) In healthy subjects, a dose as low as 81mg of aspirin has been shown to significantly suppress concentrations of colorectal mucosal PGE2.\(^18\) It is also known that aspirin induces HLA class 1 antigens which are absent on 1/3 CRC and thereby abrogate \(T\) cell receptor engagement, rendering tumors insensitive to immune mediated killing.\(^19\)
1. **Synergy with RT**: It is surprising that this cheap and relatively non-toxic therapy for which there is significant indirect evidence of enhancement of RT effect has not been studied in clinical trials. However, retrospective evidence analyzing outcome of patients on aspirin for other reasons during RT reports superior outcome (Figure 3, Restivo et al. 2015).20

![Fig. 3](image)

**Aspirin as a neoadjuvant agent during preoperative chemoradiation for rectal cancer**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Aspirin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumour Downstaging</td>
<td>44%</td>
<td>68%</td>
</tr>
<tr>
<td>Good Pathological Response</td>
<td>19%</td>
<td>46%</td>
</tr>
<tr>
<td>pCR</td>
<td>13%</td>
<td>22%</td>
</tr>
<tr>
<td>5yr PFS</td>
<td>67%</td>
<td>86% (HR 0.20)</td>
</tr>
<tr>
<td>5 yr OS</td>
<td>73%</td>
<td>90% (HR 0.60)</td>
</tr>
<tr>
<td>Distant Metastasis</td>
<td></td>
<td>HR 0.30</td>
</tr>
</tbody>
</table>

2. **Synergistic effects with PD1 inhibitor.** Preclinical models strongly support a synergistic effect of aspirin and PD-1/PDL-1 blockade antibody. Xenografted mice fed aspirin alone had little impact whereas adding anti-PD-1 therapy resulted in rapid and complete tumour shrinkage in 30%. There are no clinical studies combining aspirin and PD1 inhibition.
INTIMACCI Trial schema:

**Newly Dx,**
**T3,N1-2**
**T4, any N**
Rectal cancer

![Diagram showing treatment schema]

*1.8-2Gy x 25# (PTV_45 post-pelvis, PTV_50 primary, 9-10#/fortnight)*

Logistics:

Patients will be identified within multi-disciplinary clinics. Eligible patients who consent will be enrolled onto study prior to CRT. Patients will commence aspirin 100mg (enteric coated) orally daily and continue this until one week prior to definitive surgery.

Patients randomised to the arm containing PD1 will receive drug on D14 and D28 of CRT.

Patients will be followed until 4 weeks postoperatively for safety. A safety lead-in of 10 patients will be analysed by the Data Safety Monitoring Committee (DSMC) to ensure no excess toxicity.

Anticipated Toxicity:
PD-1 inhibitors, capecitabine and RT have broadly non-overlapping severe toxicities. Anticipated toxicities include fatigue, diarrhoea, nausea and anorexia. Additionally, patients will be monitored closely for rash, vitiligo and manifestations of autoimmune disease.

Primary endpoint:

- Pathological Complete Response (pCR) Rate
- Safety

Secondary endpoints

- Tumour regression grade
- Immune response:
  - Density of CD8(+) Tumor-Infiltrating Lymphocytes (TIL) baseline biopsy + resection specimen
MHC Class I and II expression in resected tumor and Tumor Infiltrating Lymphocytes (TILs)
PD-1 and PD-L1 expression in Tumor and TILs
Effector and Central memory T cell frequencies in TILs and Peripheral Blood Mononuclear cells (PBMCs)
TH1 : TH2 ratio
MDSCs, M2 macrophages, Tregs frequencies
Mutation landscape (whole exome), epitope mapping post radiation

pCR by PIK3CA mutation status

Current relevant studies:

-no studies on clinicaltrials.gov of aspirin + CRT for rectal cancer or Aspirin + PD1 inhibitor + CRT

Studies of CRC/RT/PD1 inhibitor:

<table>
<thead>
<tr>
<th>Tumour Stream</th>
<th>Schema</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rectal cancer</td>
<td>Phase II study Single Arm (54 patients) CRTx (Capecitabine) with Pembrolizumab 200mg D1,21,43</td>
<td>PEP: pCR SEp: Tumour CD8 Tcells, RR, DFS, OS</td>
</tr>
<tr>
<td>mCRC</td>
<td>1. Phase II trial: SBRTx + AMP-224/cyclophosphamide 2. Phase II trial: RFA + Pembrolizumab</td>
<td>PEP: RR</td>
</tr>
<tr>
<td>Anal Cancer (SCC)(CoRinTH), in development</td>
<td>Phase Ib/phase II study Single arm, 3 cohorts - Cohort 1 start anti PD-1 (200mg) at Day 29 repeat Day 50 then q21d to 6 months - Cohort 2 start anti PD-1 at day 22 repeat Day 43 then q21d to 6 months - Cohort 3 start anti PD-1 at Day 1, 22 and Day 43</td>
<td>PEP; safety/tolerability SEp: efficacy, CP, PFS, OS</td>
</tr>
</tbody>
</table>

Current studies: Other tumours +RT or SBRT with immune checkpoint inhibitor (ClinicalTrials.gov)

<table>
<thead>
<tr>
<th>Tumour</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung Cancer</td>
<td>5</td>
</tr>
<tr>
<td>Bladder Cancer</td>
<td>1</td>
</tr>
<tr>
<td>Pancreas Cancer</td>
<td>2</td>
</tr>
<tr>
<td>Metastatic Melanoma</td>
<td>1</td>
</tr>
<tr>
<td>Head and Neck Cancer</td>
<td>3</td>
</tr>
<tr>
<td>Oesophageal Cancer</td>
<td>2</td>
</tr>
<tr>
<td>Oligometastatic Breast Cancer</td>
<td>1</td>
</tr>
<tr>
<td>Metastatic Renal Cell Carcinoma</td>
<td>1</td>
</tr>
<tr>
<td>Brain cancer</td>
<td>2</td>
</tr>
<tr>
<td>Endometrial Cancer</td>
<td>1</td>
</tr>
</tbody>
</table>

Inclusion criteria:

- Signed informed consent
- Histologically confirmed rectal adenocarcinoma
- Treatment planned to be neoadjuvant CRT with capecitabine and RT
- Stage T3, N1-2, M0; T4, any N, absence of metastatic disease
- Age ≥ 18 years old
- ECOG performance status 0-2
- Life expectancy greater than 3 months
- Adequate bone marrow, liver and renal function
- Adequate contraception

**Exclusion Criteria:**
- Patients who are receiving any other investigational agents
- Patient who are already receiving aspirin, anti-platelet therapy or who require anti-platelet therapy
- Patients on anticoagulation therapy (warfarin, low molecular heparin, rivaroxaban)
- Patient with hemophilia or bleeding diathesis
- MSI+ or Lynch syndrome
- Prior treatment with PD1/PDL1
- active use of high dose steroids or other immunosuppressive agents
- History of autoimmune disease (ie SLE, Rheumatoid Arthritis, Inflammatory Bowel disease)
- History of allergic reactions attributed to compounds of similar chemical or biologic composition.
- Uncontrolled inter-current illness or concurrent severe and/or uncontrolled concomitant medical conditions that could cause unacceptable safety risk or compromise compliance.
- Patients unable to swallow orally administered medication and patients with impairment of gastrointestinal (GI) function or GI disease that may significantly alter the absorption of either study drug (e.g. ulcerative diseases, uncontrolled nausea, vomiting, diarrhoea, malabsorption syndrome).
- Patients with a past history of bowel perforation and abdominal fistula; patients with a recent history of bowel resection (within the past 12 months) and/or patients with symptoms of radiological evidence of active bowel obstruction.
- Prior radiation therapy to the rectum and/or pelvis
- Interstitial lung disease with on-going signs and symptoms

**Statistical Analysis and assumptions:**
INTIMACCI is a randomized phase II trial enrolling 86 patients (43 in each arm). The study is designed to have 80% power to detect an improvement in pCR at the time of surgery from 20% to at least 40% for either of the arms based on the Simon’s II stage design for Phase II trials (alpha =0.05), assuming that the pCR in patients having standard of care with combination chemoradiotherapy is 20%. Statistical tests to compare the treatment effect between the randomized treatment arms are not sufficiently powered and therefore not planned.

**Discussion Points:**
- EORTC GI Trials expressed interest and proposal has been forwarded to S. Tejpar to be presented at their relevant committee
- Could add in PD-1 inhibitor at Day 1
- ? need to exclude MSI pre-treatment (rare in rectal cancer with no FH)
- Interim biopsy eg immediately post CRT
- could include patients already on aspirin
Confirmation of a review of current clinical trials via WHO International Clinical Trials Registry Platform: <Search portal to identify similar studies: http://apps.who.int/trialsearch/default.aspx>
No similar trials

References: