**Title page**

# Combining conventional therapy with immunotherapy: a risky business?

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**Manuscript**

The immune system is now known to play a pivotal role in the onset and development of cancer [1,2]. The adaptive and innate immune responses are mobilized to eliminate neoplastic cells as they emerge. In some cases, elimination is not entirely successful and an equilibrium phase is established whereby the neoplastic cells enter a dormant state, side-by-side with the immune system. During this process, tumour cells can be edited and consequently escape this immunological control. Eventually, the immune system fails and uncontrolled tumour proliferation occurs. Tumours itself and/or through their interaction with the microenvironment may attract immunosuppressive cells, such as MDSC (myeloid derived suppressor cells), Treg (regulatory T cells), M2 macrophages, and others, to divert immune detection and facilitate unregulated tumour growth. Immunological evasion arises in all tumours, but the key players establishing the immune suppressive microenvironment are different per tumour, maybe even for different stages of the disease. A detailed understanding of these tumour-specific processes is gradually emerging [3] and it is essential to be aware of these as anti-cancer immunotherapy will change this environment. Failure or discontinuation of numerous clinical trials has highlighted the importance of such knowledge. Immune checkpoint inhibitors have shown limited activity in the majority of tumours and can even create accelerated cancer progression, or unexpected toxicities, when combined with other immunomodulatory drugs. This was the case with pembrolizumab, a monoclonal antibody against Programmed Death receptor-1 (anti-PD1), in multiple myeloma ((KEYNOTE 183 (NCT02576977) and KEYNOTE 185 (NCT 02579863)) and also in Adult T-cell leukemia–lymphoma [4]. Based on two recent publications in Nature Medicine it is reassuring to note that efforts are currently underway to predict sensitivity and response to immune-oncology (IO) agents prior to their initiation [5,6]. To add complexity, there is increasing evidence that

conventional treatments (chemotherapy [7-9], radiotherapy [10] and surgery [11]) also have an impact on the immune system (Figure 1). This re-enforces the importance of evaluating the immune status before commencing IO-containing combinations. Notwithstanding this lack of information, a recent review by Jessica Brown et al [8] revealed that more than 200 ongoing or planned clinical trials are registered in which IO agents are combined with chemotherapy.

This is occurring in spite of a lack of preclinical data to justify the choice of combinatorial agents. Some clinical trials have shown a better outcome when chemotherapy and immune checkpoint inhibitors are administered sequentially rather than concomitantly [12], in other studies the administration of chemotherapy after initiation of immune checkpoint inhibitors was beneficial [13]. Recently, the JAVALIN OVARIAN 100 and 200 (NCT02718417 and NCT02580058, respectively) (combining anti-PDL1 with chemotherapy in ovarian cancer) were prematurely stopped, because no significant differences in survival were obtained compared to chemotherapy in monotherapy [14,15]. Caution is needed for a possible devastating effect of combining chemotherapy with IO agents. As we have insufficient knowledge about the immune status of a patient at therapy initiation, incorrect sequencing of chemotherapy and IO agents could shorten survival. This was reported ten years ago in a first line chemotherapy study in ovarian cancer. Carboplatin and paclitaxel were administered alone or in combination with interferon gamma 1b (IFN$\gamma$1b). The study was stopped prematurely because a significantly shorter overall survival was observed in the IO containing arm ($p=0.001$). The authors only hypothesized about the cause (direct toxicity of IFN$\gamma$1b, toxicity on bone marrow leading to therapy reduction or an increase in Treg), however no immune monitoring was performed [16].

As an example, we refer to our own experience with the ID8-fLuc serous ovarian cancer mouse model [17]. We observed a shortened overall survival depending on the sequence of immunotherapy and chemotherapy (Figure 2). In the first experiment (A) dendritic cell immunotherapy was combined with carboplatin-gemcitabine, in the second (B) an anti-PD1 was combined with carboplatin-paclitaxel and an angiogenic inhibitor (DC101). The positive effects of synergistic administration (green curve experiment B) might be explained by the finding that PD-L1 expression tends to increase shortly after chemotherapy [18,19].

Shifts in immune cell composition induced by the different therapies separately or together should be considered as the cause of failure or success of combinations. This information can only be obtained by thorough preclinical work, investment in immune monitoring and assays that detect early response. Until we have that knowledge, we need to be careful in choosing the order, timing and dosage of combination therapies [9,20]. Inconsiderate decision making can result in an accidently chosen beneficial combination without understanding the underlying mechanistic reason, or it can lead to a therapeutic failure with either similar, or of greater concern, a worse outcome.

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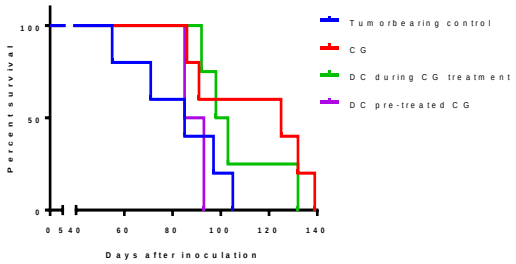
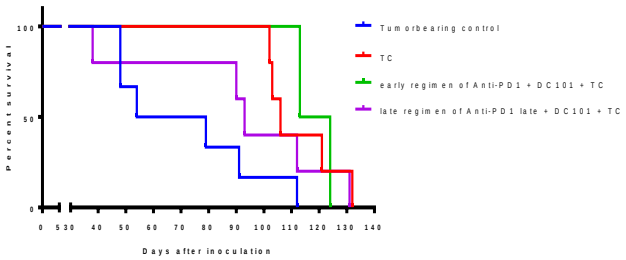
**A**


Percent survival
100
50
0
0 5 40
60
80
100
120
140
Days after inoculation
Tumorbearing control
CG
DC during CG treatment
DC pre-treated CG

**B**


Percent survival
100
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40
50
60
70
80
90
100
110
120
130
140
Days after inoculation
Tumorbearing control
TC
early regimen of Anti-PD1 + DC101 + TC
late regimen of Anti-PD1 late + DC101 + TC

Captations to figures

Figure 1. Complex interplay between conventional therapies, immune-oncology agents, cancer cells and immune cells.

Abbreviations: radiotherapy (RT), chemotherapy (CT), immune-oncology agents (IO), Indoleamine 2,3-dioxygenase (IDO), tumour growth factor (TGF), vascular endothelial growth factor (VEGF), interleukin (IL), interferon (IFN), tumour necrosis factor (TNF), cytotoxic T cell (CTL), programmed cell death 1 (PD1), cytotoxic T-lymphocyte associated protein 4 (CTLA4), T-cell immunoglobulin and mucin-domain containing-3 (TIM3), lymphocyte-activation gene 3 (LAG3), myeloid derived suppressor cell (MDSC), regulatory T cell (Treg), macrophage type 2 (M2)

Figure 2. Kaplan-Meier survival curves on combinatorial therapies, administered in the ID8-fLuc ovarian cancer mouse model

**A.** The red curve represents mice receiving carboplatin-gemcitabine (CG) only, given intraperitoneally at day 21 and day 35 after tumour inoculation (n=5). Dendritic cell (DC) immunotherapy was given subcutaneously at day 27, 34 and 41 after tumour inoculation (n=4, 1 toxicity death) (green curve) (median survival 100.5 days). The purple curve represents mice receiving DC immunotherapy at day 1, 7 and 14 after tumour inoculation (n=2, 3 toxicity deaths) (median survival 89 days). The blue curve represents tumour bearing controls, receiving no therapy (n=5). **B.** The red curve represents the group of mice receiving only carboplatin-paclitaxel (TC), given intraperiteonally at day 21 and day 35 after tumour inoculation (n=5, 1 toxicity death). The green and purple curve represent the mice receiving anti-PD1 (Clone RPM1-14, Bioceros BV, The Netherlands), DC101 (anti-VEGFR2) (1mg/kg, 2 times per week, starting from day 20) and chemotherapy with administration of anti-PD1 in an early regimen (day 20-22-24-26-28 after tumour inoculation) in case of the green curve (n=2; 4 toxicity deaths) (median survival 118.5 days) and in a late regimen (day 30-32-34-36-38 after tumour inoculation) in case of the purple curve (n=5, 1 toxicity death) (median survival 93 days). As in figure A, blue represent the untreated tumour bearing mice (n=6) (median survival 66.5 days). All murine experiments were performed in female mice, according to the EU directive 2010/63/EU and the ARRIVE guidelines.