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ABSTRACT

The genetic background and the antigenic landscape of cancer cells play a critical role in the response to immunotherapies. A high tumor antigenicity, together with an increased adjuvanticity potentially induced by a peculiar type of cell death, namely immunogenic cell death (ICD), could foster the response to immunogenic therapies. The gestational trophoblastic neoplasm (GTN) is a one-of-a-kind cancer in the oncological landscape due to its exclusive genomic makeup. The prognosis of GTN is significantly better than non-gestational trophoblastic neoplasm (nGTN). Due to its peculiar genetic inheritance, GTN potentially constitutes a singular archetype in the immuno-oncological field.

Introduction

Immunogenic cell death (ICD) is a peculiar type of cellular demise that is accompanied by the emission of danger-associated molecular patterns (DAMPs) from stressed and dying cells. Extracellular DAMPs can ligate pattern recognition receptors (PRRs) on dendritic cells (DCs) facilitating DC maturation, antigen presentation, and eventually the priming of cytotoxic T-lymphocytes (CTLs); hence, the ignition of an adaptive immune response against the antigen was originating from the dying cells [1,2]. In the past years, the relevance of ICD for the onset of anticancer immunity in response to specific antineoplastic radio and chemotherapies has been shown in suitable murine models [3]. The biological significance of this kind of cell death and its relevance in promoting anticancer immunosurveillance has been comprehensively reviewed elsewhere [4,5]. Furthermore, neoplastic cells undergoing ICD can successfully vaccinate syngeneic mice against re-challenge with living malignant cells of the same antigenic origin. This peculiar type of cell death is accompanied by transcriptional arrest, the autophagy-dependent release of ATP, ER-stress-mediated exposure of calreticulin, the exodus of HMGB1, and type I interferon responses have been found to characterize the onset of ICD. These hallmarks of ICD have been employed to screen large compound libraries with suitable biosensors. Using such workflows, actinomycin D was recently found to induce ICD of cancer cells *in vitro* and to elicit anticancer immunity *in vivo* [6].

Focus on gestational choriocarcinoma

Results from the clinical management of gestational choriocarcinoma, a rare female neoplasm commonly treated with an optimized polychemotherapy regimen, may support the possible significance of ICD in inducing the treatment response. The choriocarcinoma can develop from germ cells of the ovary (non-gestational choriocarcinoma) or from the trophoblast (gestational choriocarcinoma). The former arises from pluripotent germ cells, while the gestational subtype can occur following a hydatidiform mole, a normal pregnancy, or an abortion [7,8]. Even though precise estimates of the incidence of these rare neoplasms are difficult to be determined (especially for the two distinctive subtypes), the incidence of choriocarcinoma is estimated to be of 1:40,000 pregnancies and 1:40 hydatidiform moles in Europe and North America [9].

Sometimes, the gestational and non-gestational subtypes can apparently exhibit indistinguishable histological and clinical features. However, only the gestational choriocarcinoma has DNA of paternal origin in its genome. Indeed, in childbearing women, the gestational choriocarcinoma is differentiated from the (non-gestational) germ-cell

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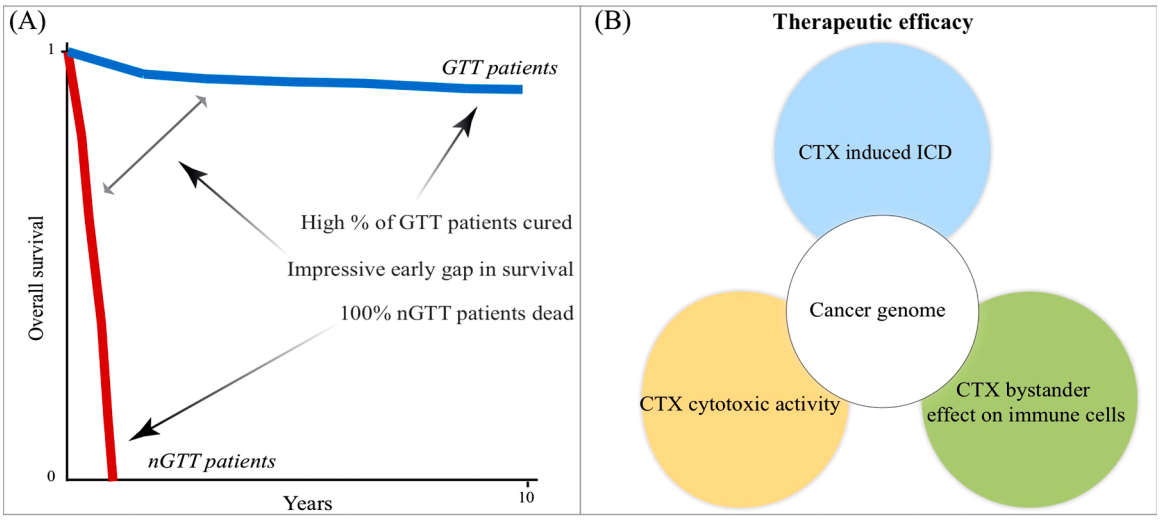

Fig. 1. **(A)** Figurative representation of the Kaplan–Meier curves to underline the large gap in survival between patients with gestational trophoblastic neoplasm (GTN) versus non-gestational trophoblastic neoplasm (nGTN) drafted on the basis of survival data reported by Alifrangis et al. JCO 2013 [15]. **(B)** Hypothetic hallmarks for therapeutic activity in GTN.

subtype, by detecting the presence of paternal DNA in tumoral cells [10,11]. Consequently, gestational choriocarcinoma is a unique neoplasm in its genetic background. As it stands, it could be defined as a semi-allograft tumor (or allograft tumor in case of a fully paternal derived genome).

According to the International Federation of Gynecology and Obstetrics (FIGO) prognostic score, patients with gestational choriocarcinoma or other gestational trophoblastic neoplasms (GTN) can be differentiated into low- or high-risk groups [12]. Single-agent chemotherapy regimens (either methotrexate or actinomycin D) are widely used in low-risk GTN patients. On the other hand, high-risk GTN is usually treated with combination chemotherapies such as EMA-CO (etoposide, methotrexate, actinomycin D, cyclophosphamide, and vincristine) or EP-EMA (cisplatin, etoposide, methotrexate, and actinomycin-D); the latter in case of recurrent disease or if primary resistance to EMA-CO occurs [7,10,13,14].

Thanks to the advent of these powerful combination chemotherapies, the survival rate of high-risk GTN has significantly improved during the past decades [13]. Remarkably, the prognosis of the gestational subtypes is significantly better than non-gestational ones (nGTN) as reported by retrospective series (where patients were screened by genetic analysis in case of atypical disease course), and, contrary to what happens in patients with nGTN, chemotherapy is generally curative for patients with GTN (Fig. 1a) [15,16].

As discussed above, the presence of paternal genes in the genome of GTN cells constitutes a peculiarity. This condition probably results in the expression of exogenous antigens (derived from the paternal genetic background), thus making this malignancy extremely immunogenic. In this context, it is tempting to speculate (yet remains to be formally proven) that the better survival of GTN patients may partially rely on bystander immunological effects triggered by an optimal combination of chemotherapic drugs. Indeed, methotrexate, actinomycin D, cyclophosphamide, and vincristine are highly immunogenic, while etoposide, as well as cisplatin, is a potent cell death inducer [2,6,17], again suggesting a role of ICD induction in the clinical outcome of GTN therapy.

Another suggestion that could corroborate this translational biological speculation derive from data on the clinical benefit obtainable with immunotherapy in the few patients who failed to respond to EMACO.

Clearly, our hypothesis awaits experimental confirmation by dedicated preclinical models and immunopeptidome/tumoral-neoantigens data from clinical series. Other clinical evidence on beneficial therapy-induced immunological effects has already been reported in other malignancies such as lung cancers and hematological malignancies [18]. Hence, the "off-target activity" of conventional anticancer drugs, as well as possible bystander effects on the immune system, deserves further investigation (Fig. 1b).

Discussion

The genetic background and therefore the antigenic landscape of the malignant cells play a decisive role in shaping the therapeutic response to immunotherapeutic regimen. Highly immunogenic neoplasms, such as MMR-deficient cancers, can be optimally treated with immunotherapies given their capacity to reinstate anticancer immunosurveillance [19].

The same concept might apply to GTN, which, due to its genetic inheritance, provides the ideal premises for eliciting anticancer immunity when stimulated for antigen presentation. It is therefore tempting to speculate that the probably high antigenicity of GTN, together with an increased adjuvanticity [20] induced by ICD inducing agents (such as actinomycin D) could orchestrate the response to therapy in this rare neoplastic disease. This hypothesis might partially explain the high rate of success with durable remission that can be achieved by combination chemotherapeutic treatment of GTN. This supposition needs experimental validation yet already stimulates questions on the origin of the immunologically relevant antigens (in the context of GTN). Lastly, this evidence strengthens the rationale for the investigation of immunomodulating drugs in combination with immunotherapies.

CRediT authorship contribution statement

G. Frega: Conceptualization, Writing – original draft, Writing – review & editing, Supervision. **O. Kepp:** Conceptualization, Writing – original draft, Writing – review & editing, Supervision. **D. Turchetti:** Writing – review & editing. **A. Rizzo:** . **M.A. Pantaleo:** Writing – review & editing. **G. Brandi:** Conceptualization, Writing – review & editing.

Declaration of Competing Interest

Frega G. declares that he has no conflict of interest. Kepp O. declares that he has no conflict of interest. Turchetti D. declares that she has no conflict of interest. Pantaleo M.A. declares that she has no conflict of interest. Brandi G. declares that he has no conflict of interest.

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Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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