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EDITORIAL

Immunogenic stress induced by local anesthetics injected into neoplastic lesions

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The mechanical excision of tumors by oncological surgery, as well as the effractions imposed on vascular and lymphatic vessels, can promote the dissemination of residual tumor cells to distant organs with the consequent formation of secondary metastatic lesions.[1] Moreover, pain and inflammation, which are often elicited by surgical procedures, cause systemic adaptations such as the production of immunosuppressive glucocorticoids and vascular endothelial growth factor, thus affecting leukocyte chemotaxis and favoring neoangiogenesis, respectively. Local anesthetics that are currently used in clinical routine for their analgesic and anti-inflammatory properties can minimize the release of corticoids and catecholamines during surgical procedures. Moreover, several retrospective clinical trials recently suggested that the use of local anesthetics associated with general anesthesia during oncological surgery improves disease outcome and overall survival.[2] Intrigued by these premises, we addressed the question if local anesthetics directly affect neoplastic cells and whether they promote an anti-tumor immune response that might explain their positive impact on cancer prognosis.

Accumulating preclinical evidence suggests that local anesthetics provoke direct cytotoxic effects, leading to the inhibition of proliferation, survival and migration of various malignant cell types. Several mechanisms have been implicated in this process such as the impairment of cyclin-dependent kinase activity, the suppression of DNA methylation, the induction of mitochondrial damage and dysfunctional ion transport.[3] Driven by these findings, we studied the mode of cell death that is induced by local anesthetics, finding that apoptotic as well as necrotic signaling pathways are ignited in a dose- and time-dependent manner. Moreover, treatment with clinically relevant concentrations of the most frequently employed local anesthetics induced traits of immunogenic cell death (ICD) such as the release of ATP and the exodus of high-mobility group box 1 protein (HMGB1) into the extracellular space.[4,5] This phenomenon was preceded by premortem cellular stress routines such as the induction of autophagy and the phosphorylation of eIF2alpha, which is known to be involved in immunogenic death as well as in the release of pro-inflammatory cytokines.[6,7] In contrast to *bona fide* ICD-inducing chemotherapeutics such as mitoxantrone, oxaliplatin or microtubule-targeted poisons, local anesthetics were unable to induce the exposure of calreticulin at the plasma membrane, where it commonly acts as an "eat-me" signal for dendritic cells. Local anesthetics also failed to inhibit DNA-to-RNA transcription, which has recently be described as a prominent upstream signal causing ICD.[8,9] Nevertheless, local anesthetics regulate adaptive immune response by promoting the phagocytosis of dying tumors by bone marrow-derived dendritic cells (BM-DCs) *in vitro* and by decreasing the growth of palpable tumors established in immunocompetent mice, if injected intratumorally.[10] Interestingly, local anesthetics failed to promote anti-tumor effects in immunodeficient mice, as well as against tumors that were rendered unable to mount autophagic or ER stress responses due to the inactivation of *ATG5* and *EIF2AK3*, respectively. Moreover, local treatment with local anesthetics induced the infiltration of tumors by $CD4^+$ and $CD8^+$ T cell effectors, but diminished the local recruitment of immunosuppressive T regulatory cells.[4]

As described previously, the efficacy of conventional anti-neoplastic treatments may be potentiated by immunostimulatory agents.[11,12] Thus, we further investigated if the combination of local anesthetics with adjuvant stimulation may restore a full-blown ICD response and may potentiate antitumor immunity.[13,14] Indeed, the local co-injection of recombinant calreticulin together with local anesthetics further improved the anticancer effect. The use of local anesthetics combined with sequential systemic immune checkpoint blockade even allowed to completely and durably eliminate some established cancers,[4] in which case mice were protected from rechallenge with the same cancer, indicating that they had developed immunological memory.

Altogether, our preclinical findings highlight that the use of local anesthetics causes "immunogenic stress" rather than full-blown ICD. Thus, local anesthetics may be classified among the non-viral oncolytic therapies, which upon intra-tumoral injection elicit immunogenic physical and chemical damage.[15–17] Irrespective of their classification, local anesthetics trigger the phagocytosis of cancer cells and the engulfment of tumor antigens by DC, followed by the recruitment of immune

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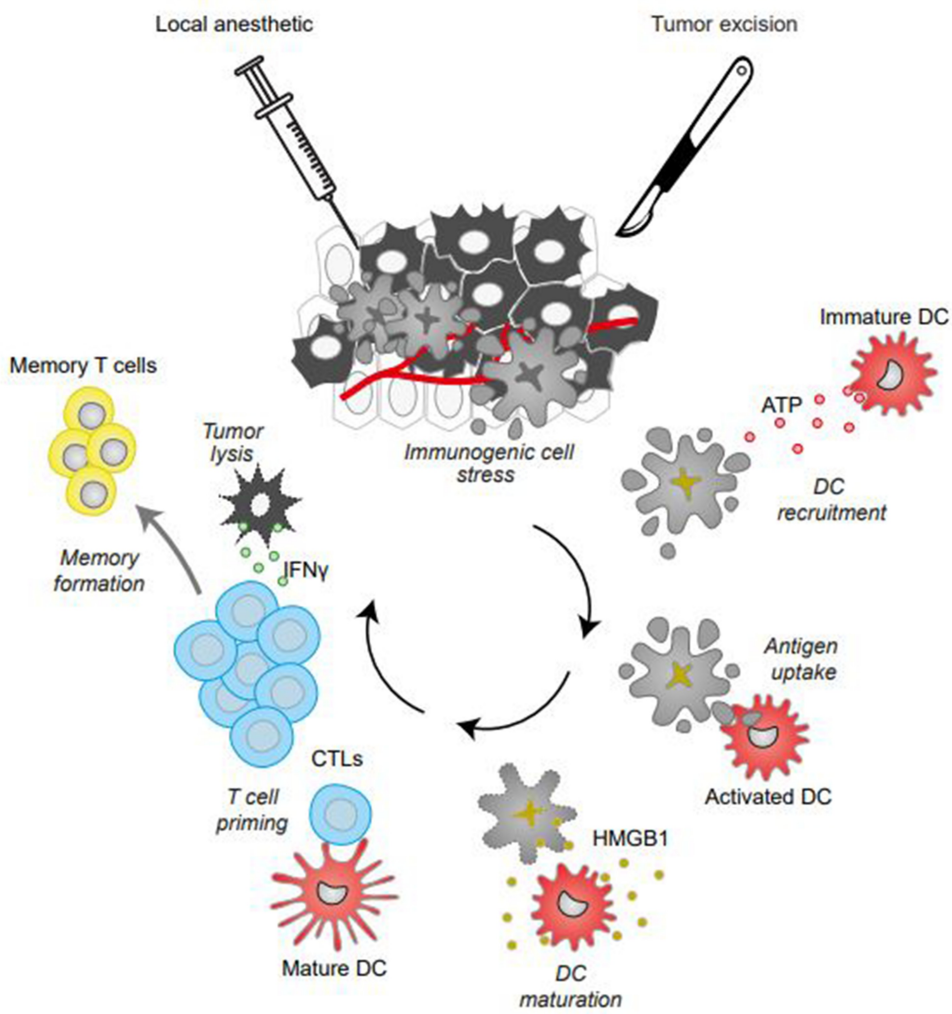

Figure 1. Oncolytic effects of local anesthetics. Local anesthetics employed at clinically relevant concentrations induce cell death. This effect is preceded by premortem autophagy and endoplasmic reticulum stress and accompanied by the release of danger-associated molecular patterns (DAMPs) such as ATP and high-mobility group box 1 protein (HMGB1), facilitating the recruitment of dendritic cells (DCs) for antigen presentation and T lymphocyte priming, altogether eliciting adaptive anticancer immune responses, tumor lysis by cytotoxic T lymphocytes and the generation of durable immune memory.

effectors into the tumor bed (Figure 1). Several strategies associating local anesthetics with immunostimulatory agents may be envisaged to further boost the cancer-immunity cycle.

In sum, beyond their role in pain control, local anesthetics may be repurposed as antineoplastic co-treatments to avoid disease recurrence and improve overall survival because of their capacity to induce immunogenic stress. There is an urgent need to design randomized controlled trials that support this exciting hypothesis that ultimately would change clinical practice in onco-anesthesia.

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Data availability statement

The data that support the findings of this editorial are available at doi:10.1136/jitc-2021-004151. PMID:35483744. https://jitc.bmj.com/content/10/4/e004151.

Declaration of interest statement

OK is a scientific co-founder of Samsara Therapeutics. GK has been holding research contracts with Daiichi Sankyo, Eleor, Kaleido, Lytix Pharma, PharmaMar, Samsara, Sanofi, Sotio, Vascage and Vasculox/Tioma. GK is on the Board of Directors of the Bristol Myers Squibb Foundation France. GK is a scientific co-founder of EverImmune, Osasuna Therapeutics, Samsara Therapeutics and Therafast Bio. GK is the inventor of patents covering therapeutic targeting of aging, cancer, cystic fibrosis and metabolic disorders.

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