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Chemo 2.0: Programmed Necrosis Agents for Broader and More Effective Cancer Immunotherapy

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ABSTRACT

Despite the recent breakthroughs in cancer immunotherapies, two major challenges remain in modern oncology in further advancing patient survival benefits-chemo-resistance development and low patient response rates to immune checkpoint inhibitors. Two new approaches at solving these problems via induction of programmed necrosis in target tumor were recently disclosed, one using MLKL-mRNA that targets necroptosis induction in tumors, and the other using peptide- assisted zinc compound that targets parthanatos induction in tumors. This mini-review summarizes potential advantages of these new approaches, their current caveats, and their identified translational challenges ahead.

Keywords: Cancer, Immune therapy, Chemotherapy, Immunity, Necrosis, Necroptosis, Parthanatos

INTRODUCTION

Chemotherapy to-date remains as the most widely prescribed anti-cancer regimen, as it has been historically proven to provide highly effective treatment benefits against many cancer types with or without added immune checkpoint inhibitors (ICI) [1]. Naturally, innate and treatment-induced chemo-resistance development was reported to be responsible for 90% of failed cancer treatment cases, attracting heated research interests for decades [2]. Among different approaches studied on the topic of defeating chemo-resistance, the latest developments include programmed necrosis agents (PNA) via necroptosis or parthanatos that uniquely offer additional benefits of amplifying the immunotherapeutic actions of the immune checkpoint inhibitors (ICI) via strengthened immunogenic tumor deaths and immune modulation [3-5]. This article briefly reviews their drug mechanisms that nullify chemo-resistance modes, their disclosed immunotherapeutic benefits, and potential strengths/caveats going forward.

Five chemo-resistance development mechanisms have been identified to-date, which include the suppression of p53-caspases-mediated apoptosis, altered drug metabolism leading to drug inactivation, enhanced DNA-repair, drug target changes, or target gene amplification or epigenetic changes of similar effects. In any given cancer, the chemo-resistance may occur innately or treatment-induced, involving any number of combined resistance modes per cancer progression that frequently lead to multi-drug resistance [2]. Of a particular challenge to conventional chemotherapies that almost exclusively relied on p53-caspases-mediated apoptosis, over 50% of cancer cases are found with mutated and dysfunctional p53 that result in suppressed apoptosis and induction of other drug resistance mechanisms [6-10]. In this sense, the PNA approaches that circumvent the entire p53 and caspases apoptosis machineries confer great starting advantages in combating the chemo-resistant cancers. Briefly, necroptosis is an immunogenic programmed necrosis that is mediated by RIPK3 with MLKL as the effector enzyme that leads to membrane rupture [11]. Parthanatos, on the other hand, is an immunogenic programmed necrosis that is mediated by hyperactivity of PARP1 with nuclear- translocated AIF as the effector enzyme that leads to chromatinolysis.

A leading development on necroptosis induction for anti-cancer use involves MLKL-mRNA as the active therapeutic agent [4]. An important advantage of this approach is that it directly upregulates the effector enzyme step of the pathway, eliminating potential resistance development risks from downstream genetic or epigenic cell responses. Of particular interest, this approach was demonstrated with clear immunotherapeutic effects involving potent T-cell initiation and synergism with an anti-PD1 treatment that led to tumor eradication, metastasis prevention, and relapse-prevention from tumor re-inoculation after initial surgical removal in murine cancer models. In a similar model study using RIPK3-mRNA, the same group also demonstrated that the *in vitro* necroptosized murine cancer cells, but not the cells killed by accidental necrosis, conferred *in vivo* vaccination benefits against the cancer model involving dendritic cell priming and subsequent T cell immune responses. Current caveats of this development, on the other hand, include low direct cytotoxicity *in vitro*, lack of the screening data supporting its broad effectiveness versus different cancer types, lack of the data supporting its effectiveness against human cancer in humanized immunity animal models, and the use of electroporation-mediated direct tumor injection of the MLKL-mRNA as its administration method. While recent clinical success of COVID19-mRNA vaccines involving liposome-encapsulated mRNA serves as promising signs of its translation, the added requirement of enough MLKL-mRNA delivery to the tumor tissue for sufficiently strong cancer necroptosis induction in systemic use setting may be a challenge for this approach.

The leading development on parthanatos induction for anti-cancer use involves a biodegradable modified peptide-assisted zinc (II) named 010DS-Zn as the active therapeutic agent, wherein the modified Ɣ-polyglutamate peptide acts as the amplifier of the zinc (II)'s ability to induce parthanatos in tumor upon its accumulation and digestion [5]. An interesting aspect of this new approach is that it transforms one of the safest and the most common biological metal zinc (II) into a cytotoxic agent, achieving *in vitro* IC505d values well below 1 µM Zn. For reference, basal serum zinc level in healthy men was reported at 22.33 ± 6.42 µM Zn and 21.72 ± 6.42 µM Zn in healthy women among adults [12]. An important advantage of this approach is that all metabolites of the agent such as zinc (II), glutamates, PEG, and pyrithiones have been historically documented for their safety and widespread use. This approach demonstrated evidence of consistent parthanatos induction and efficient cytotoxicity (>99% kill) on all 53 of the 53 low passage human patient-derived solid cancer fragments *ex vivo*, which characteristically demonstrated intense chromatinolysis versus their apoptosis control (10% DMSO). And alike the counterpart development in necroptosis, *in vivo* monotherapy using 010DS-Zn also demonstrated significant tumor growth suppression effect versus multiple murine tumor models, which were characterized by robust CD4 and CD8 T cell anti-tumor immune initiations and M2-like macrophage reduction in the collected tumors. Current caveats of the parthanatos-induction approach using 010DS-Zn include lack of the data supporting its synergism with immune checkpoint inhibitors, lack of replicating *in vivo* studies, lack of the data demonstrating its efficacy against human cancer in humanized immunity animal models, and identified issues with its poor pharmacokinetic profile requiring biodistribution-modifying liposome formulation development. Although its efficient parthanatos-inducing cytotoxicity *in vitro* suggests favorable translational odds upon its formulation into liposomes, it's *in vivo* effectiveness and potential cancer treatment potentials requires further scrutinizing studies.

A promising pharmacological aspect to note from both MLKL-mRNA and 010DS-Zn developments is that neither agents are small molecular organic compounds, which raises possibilities of their application in previously treated patients with known cross-resistance to conventional chemotherapies. Briefly, cross-resistance in oncology is a common phenomenon wherein a cancer acquires drug resistance to multiple drugs after the first drug treatment, often leading to multi-drug resistance in cancer and subsequent treatment failures. And as the majority of conventional chemotherapeutic agents are small organic molecules, the most common mode of cross resistance development after a conventional chemo treatment involves the overexpression of ATP-dependent small molecular drug ejection pumps such as PgP, BCRP, or MRP1 that can collectively make a cancer resistant to most widely-used chemo agents including paclitaxel, doxorubicin, mitoxantrone, topotecan, and cisplatin, just to name a few [13]. Given the fact that the large molecular compounds MLKL-mRNA and 010DS-Zn cannot be substrates to the small molecular drug ejection pumps, the new PNA compounds respectively targeting necroptosis and parthanatos provides clear rationale of their potential treatment utilities in previously chemo-treated cases with known multi-resistance to

conventional chemotherapeutic agents.

A common development challenge between both MLKL-mRNA and the 010DS-Zn is the requirement of successful liposome formulation with preferential targeting into solid tumor tissues to enable their systemic use. While liposome coating formulation has been successfully used and globally accepted over the course of the mRNA-based COVID19 vaccine deployment, its use in oncology is still in its early stage with few FDA-approvals to date, including verteporfin-liposome (2000), vincristine-liposome (2012), daunorubicin-liposome (1996), cytarabine-liposome, doxorubicin-liposome (1995), and doxorubicin-bortezomib-liposome (2007) [14]. As the changes in the physical parameters of the liposome coating implicate deterministic changes in the biodistribution and pharmacokinetic of the payloads, optimization of the pharmaceutical effectiveness and the safety profile of the future MLKL-mRNA and 010DS-Zn formulation products will require careful designs and rigorous *in vivo* tests [14]. Of particular importance, the choice of using non-PEGylated liposome (conventional) or PEGylated liposome during such formulation may potentially confer direct immunomodulatory activity against phagocytic immune cells such as macrophages or dendritic cells. Accordingly, exhaustive immune characterization will be needed during the *in vivo* testing of the liposome formulations of the MLKL-mRNA and 010DS-Zn.

In context of the intense current interests on the discovery of drug combination therapies for improving the reliability and the effectiveness of ICI, it is worth noting that the induction of immunogenic cancer death with maximum tolerable chemotherapy emerged as one of the most successful regimen and subsequently received FDA indication approvals [for a more detailed review on this topic, refer to [13]]. Briefly, addition of the ICI agent pembrolizumab to the carboplatin-pemetrexed regimen led to 44% reduction in the risk of death (OS–HR=0.56, CI:0.32-0.95) against non-squamous NSCLC (Phase 2 KEYNOTE-021 trial), while adding pembrolizumab to the conventional fluorouracil+cisplatin treatment regimen for locally advanced or metastatic gastrophaegeal junction (GEJ) carcinoma reduced the risk of death by 27% (OS-HR=0.73, CI:0.62-0.86) (Phase 3 KEYNOTE-590 trial) [14,15]. Unfortunately, stringent toxicity of the conventional cytotoxic chemotherapy proved to be a complicating factor in widening the applicability of the chemo-ICI combination treatment, as some combinations increased the incidence of severe (grade 3-5) treatment related adverse events (TRAE), and in some cases abrogated the ICI effectiveness via immune-toxicity [1,16]. In this light, the newly developing PNAs such as the necroptosis-targeting MLKL-mRNA or the parthanatos-targeting 010DS-Zn offer good theoretical frameworks to further advance the clinical success of the chemo-ICI combination regimens by enabling more consistent cytotoxicity directed against cancer, strengthened immunogenic signals from the regulated necrosis in the target cells, and better immune-modulation in the tumor microenvironments involving dendritic cells and/or macrophages [4,5]. Future translational studies on these PNAs that peruse *in vivo* efficacy screening versus human cancers in humanized immunity animals, and those that investigate the synergism with leading ICIs such as pembrolizumab or nivolumab in the same models would be of great interests to both medical and industrial research communities alike.

CONFLICTS OF INTERESTS

JFC is a director and a shareholder of Xylonix PTE, LTD.

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