ONCOIMMUNOLOGY
2020, VOL. 9, NO. 1, e1746172 (2 pages)
https://doi.org/10.1080/2162402X.2020.1746172

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POINT-OF-VIEW

RRx-001, a first-in-class small molecule inhibitor of MYC and a downregulator of CD47, is an "erythrophagoimmunotherapeutic"

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ABSTRACT

The main mechanism of action of RRx-001, a pharmaceutically unprecedented *sui generis* Phase 3 small molecule that is derived from the aerospace industry, is clarified. RRx-001 has demonstrated anticancer activity through antiangiogenic, immune, epigenetic, antioxidant, apoptotic and nitric oxide (NO) pathways, resulting in its pleiomorphic description as an antiangiogenic/vascular normalizer.

ARTICLE HISTORY
Received 4 February 2020
Revised 4 April 2020
Accepted 11 February 2020

KEYWORDS
Immunotherapy; small molecule; erythrophagoimmunotherapeutic; MYC; CD47; hemoglobin; tumor associated macrophage

We have written this *Point-of-view* article to clarify the main mechanism of action of RRx-001, a pharmaceutically unprecedented *sui generis* Phase 3 small molecule that is derived from the aerospace industry. RRx-001 has demonstrated anticancer activity through antiangiogenic, immune, epigenetic, antioxidant, apoptotic and nitric oxide (NO) pathways, resulting in its pleiomorphic description as an antiangiogenic/vascular normalizer,[1] a reactive oxygen species (ROS)-inducer,[2] a pro-apoptotic,[3] a p53 inducer, an epi-immunotherapeutic[4] and a nitric oxide donor.[5,6]

Based on the most recent clinical and preclinical data, RRx-001 is perhaps most accurately characterized as an erythrophagoimmunotherapeutic in cancer, that is, a hemoglobin (Hb)-conjugated small molecule, which inhibits MYC, downregulates CD47, a ubiquitous antiphagocytic signal on tumors, and targets tumor-associated macrophages. On intravenous infusion, RRx-001 selectively partitions into red blood cells (RBCs) and binds irreversibly to hemoglobin beta cysteine 93 (βCys93). In the process of binding to βCys93, RRx-001 not only displaces nitric oxide from βCys93 but also accelerates the deoxyhemoglobin-mediated conversion of nitrite to nitric oxide under hypoxic conditions,[7] leading to the previous characterization of RRx-001, as an NO donor.

These RRx-001-bound red cells, which travel with the blood flow to the tumor where they obstruct the hypoxic microvasculature due to their increased rigidity, undergo phagocytosis by tumor-associated macrophages or TAMs. Therefore, RRx-001, as a small molecule bound to red blood cells, specifically targets the reticuloendothelial cells of the tumor to induce MYC inhibition, CD47 downregulation and M1 polarization of M2 anti-inflammatory, pro-tumor TAMs.[8]

Unlike other small molecule nitroxyl (HNO) or nitric oxide (NO) donors,[9] such as Angeli's salt, Diazeniumdiolates or NONOates and nitrate esters like nitroglycerin, which tend to release NO spontaneously or enzymatically under aerated rather than hypoxic conditions, RRx-001-mediated NO generation is a byproduct of the conjugation of RRx-001 to hemoglobin and derives specifically from the red cell itself rather than the small molecule.

For this reason, we believe that RRx-001, which is structurally unique and not a derivative of an NO donor class, should be assigned its own suffix or stem proper to a first-in-class "erythrophagoimmunotherapeutic" agent. Moreover, the term, NO donor, which implies a primary anticancer role for nitric oxide generation, may lead to the misuse of RRx-001 since, in fact, NO generation is one of its many *submechanisms* including antiangiogenesis, epigenetic modification, ROS and apoptosis induction that are secondary or even tertiary to macrophage polarization. Several new publications that provide clinical and preclinical evidence of the centrality of CD47 downregulation and macrophage polarization as a *sine qua non* for the anticancer activity of RRx-001 are forthcoming.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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