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ABSTRACT

Introduction: According to Hanahan and Weinberg, cancer manifests as six essential physiologic hallmarks: (1) self-sufficiency in growth signals, (2) insensitivity to growth-inhibitory signals, (3) evasion of programmed cell death, (4) limitless replicative potential, (5) sustained angiogenesis, and (6) invasion and metastasis. As a facilitator of these traits as well as immunosuppression and chemoresistance, the presence of tumor-associated macrophages (TAMs) may serve as the seventh hallmark of cancer. Anticancer agents that successfully reprogram TAMs to target rather than support tumor cells may hold the key to better therapeutic outcomes.

Areas covered: This article summarizes the characteristics of the macrophage-stimulating agent RRx-001, a molecular iconoclast, sourced from the aerospace industry, with a particular emphasis on the cell-to-cell transfer mechanism of action (RBCs to TAMs) underlying its antitumor activity as well as its chemo and radioprotective properties, consolidated from various preclinical and clinical studies.

Expert opinion: RRx-001 is macrophage-stimulating agent with the potential to synergize with chemotherapy, radiotherapy and immunotherapy while simultaneously protecting normal tissues from their cytotoxic effects. Given the promising indications of activity in multiple tumor types and these normal tissue protective properties, RRx-001 may be used to treat a broad spectrum of malignancies, if it is approved in the future.

1. Introduction

RRx-001, a first-in-class immunostimulatory agent (see Table 1), which contains a novel dinitroazetidine pharmacophore, was in-sourced from a major aerospace contractor, ATK-Orbital, on the initial assumption that it would deplete cellular glutathione, the principal scavenger of reactive oxygen/nitrogen species, thereby inducing the formation of free radicals and sensitizing hypoxic cells to the ionizing effects of radiation. This assumption was based on evidence of (1) covalent binding of RRx-001 to reduced glutathione and (2) the presence of geminal dinitro-groups in the four-membered azetidine ring of the molecule; these dinitro-groups were hypothesized to decompose in vivo under hypoxic conditions, producing the gaseous free radical, nitric oxide. However, as it turned out, the mechanism is more involved than reduced thiol depletion or nitric oxide radical-induced radiosensitization, even though reactive oxygen and nitrogen species were subsequently found to play prominent roles as M1 activators of tumor-associated macrophages (TAMs), which, in turn, mediate oxidant injury to the cancer cells and the cancer stroma.

In addition to radiosensitization, RRx-001 demonstrated potent anticancer activity as a single agent [1]; significant cytotoxic enhancement was also present in combination with standard chemotherapy and immunotherapy through depletion of cancer stem cells (CSCs) and activation of p53 and Nrf2 pathways [1–3]. Moreover, RRx-001 has shown evidence of in vivo radio- and chemoprotection; in fact, the Armed Forces Radiobiology Research Institute is currently researching RRx-001 as a radioprotectant in case of a nuclear emergency.

RRx-001 is also under clinical investigation in several Phase II studies both as monotherapy and as a combination therapy with traditional chemotherapy, immunotherapy, and radiotherapy for the treatment of refractory solid tumors. This review briefly summarizes the current understanding of the chemistry and the mechanism of action of RRx-001, which has demonstrated a new paradigm of TAM reeducation and prosensitization, a hybrid term...
referring to tumor sensitization and normal tissue protection. The results of the completed Phase I study and ongoing Phase II studies are mentioned, in which, to date, RRx-001 has sensitized tumors to the effects of chemotherapy and radiation without systemic side effects [4].

2. Properties of RRx-001

2.1. Chemical structure

Like its closest chemical relative, the propellant, 1,3,3 trinitroazetidine (TNAZ) [5], (Figure 1(a)), RRx-001 is a readily synthesized [6], highly ring-strained nitroalkane possessing geminal dinitro groups. In RRx-001, also known by its chemical acronyn, ABDNAZ, the dinitroazetidine scaffold from TNAZ displays a sulfhydryl-selective α-bromoacetyl functionality [7] (Figure 1(b)) that forms stable adducts with reduced glutathione, a conserved cysteine 93 beta residue of hemoglobin, and, to a lesser extent, cysteine, via displacement of the bromide atom [8]. Analogs with α-chloro or α-iodo groups in place of the bromine resulted in decreased cytotoxicity, indicating the importance of relative electrophilic reactivity [9].

Multiple lines of evidence indicate that metabolites of RRx-001 cross the blood–brain barrier: (1) in an ADME study with 14C-labeled RRx-001, radioactivity was detected in the CNS [8]; (2) in an ongoing Phase I/II trial acronymed BRAINSTORM, a significant reduction in the Dynamic contrast-enhanced – magnetic resonance imaging (DCE-MRI)-derived volume transfer constant, Ktrans, has been demonstrated in multiple patients after one dose of single agent RRx-001, indicative of reduced vascular permeability; and (3) the combination of RRx-001 with artemisinin, in an experimental cerebral malaria model, synergistically decreased animal mortality, neurocognitive sequelae, cerebral vascular changes, and inflammatory cell accumulation in brain microvessels [10].

2.2. Metabolism of RRx-001 in mammalian cells

On intravenous injection, RRx-001 binds irreversibly and with high affinity and selectivity to specific electron-rich nucleophilic thiol groups on (1) the beta-93 Cys residue of hemoglobin to form adducts that persist for the lifetime of the red blood cell and (2) reduced glutathione, where the adducts are rapidly excreted through the kidneys [8]. RRx-001 binding at the beta-93 Cys residue results in conformational Hb changes, leading to an increase in oxidative stress within the red blood cell, and hemoglobin denaturation with deposition of denatured hemoglobin (hemichrome) on the membrane and the development of various membrane effects that are the consequence of oxidative stress. In addition, hemoglobin acts as a nitrite reductase converting nitrite to nitric oxide under conditions of low oxygen and pH [11]. Covalent binding of RRx-001 to the exposed beta-93 cysteine residues on hemoglobin tonically increases the rate of nitrite reduction to NO under hypoxia compared with unmodified red blood cells in vitro [11]. In this way,
covalently bound RRx-001 drives a catalytic hemoglobin-mediated production of nitric oxide under deep hypoxia [12,13], a defining characteristic of tumors, which further accelerates denaturation of hemoglobin and loss of heme. RRx-001 is currently administered intravenously. However, oral activity was demonstrated in unpublished preclinical animal experiments.

2.3. Pharmacokinetics (PK)

RRx-001 is quickly metabolized, and as a result of its very rapid rate of disappearance, the RRx-001-GSH adduct represented exposure to the parent compound in the Phase I clinical trial. Using a validated analytical method [14] to detect RRx-001-GSH, the adduct was found to have a terminal half-life of approximately 30 min and a volume of distribution which parallels the extracellular fluid. AUC and Cmax were mostly dose proportional up to 55 mg/m² [4,15].

2.4. Cellular Trojan Horse method of tumor-specific delivery

After infusion of RRx-001, a four-step series of events occurs (see Figure 1(c)):

1. RRx-001 binds to hemoglobin in red blood cells, which alters RBC membrane properties
2. RRx-001-modified RBCs adhere to hypoxic tumor vascular endothelial cells
3. Akin to a Trojan Horse these RBCs are internalized (phagocytized) by the TAMs, which M1 activates them
4. These M1 activated macrophages produce and release reactive oxygen and nitrogen species and immunogenic cytokines and chemokines as well as present tumor antigens to initiate adaptive T-cell immunity [16]

This RBC-‘guided’ Trojan Horse method of delivery likely accounts for the lack of collateral systemic toxicity since unbound RRx-001 is never exposed to normal tissues.

2.5. Mechanism of action

The anticancer mechanism of action of RRx-001 resembles a signal transduction pathway, composed of a trigger event, the binding of RRx-001 (‘the ligand’) to the β-93 residue on hemoglobin (‘the receptor’), as described earlier, in a nearly exclusive and highly specific fashion. This initial ‘signal’ precipitates a cascade of oxidative reactions in the red blood cell, which propagates to the tumor, resulting in a domino-like chain of events: alteration and ‘scrambling’ of the red cell membrane ⇒ microvascular occlusion and RBC internalization by the TAMs ⇒ vascular normalization and improved blood flow ⇒ epigenetic changes ⇒ immune activation ⇒ chemo and radiosensitization (see Figure 1(c)).

3. Clinical trials

3.1. Safety profile

In the Phase 1 clinical trial, the most typical and, in fact, really the only adverse event related to the administration of RRx-001, occurring in >80% of patients and mostly grades 1 and 2, was transient local pain at the site of injection that resolved spontaneously within seconds after the infusion was complete [4]. Notably, however, likely due to the RBC-based tumor-specific mechanism of delivery, described earlier, RRx-001 was devoid of systemic toxicity at all of the doses tested clinically. Thus, the common chemotherapy toxicities of alopecia, myelosuppression, diarrhea, mucositis, nausea, vomiting, and even the toxicities of the so-called targeted agents were not observed with RRx-001, which suggests a low potential for unfavorable drug–drug interactions. The absence of systemic toxicity is consistent with the narrow specificity, selectivity, and rapidity of its binding to hemoglobin and glutathione (in contrast to the off-target interactions with most drugs) and subsequent transport via the red blood cell to the hypoxic tumor vasculature.

Due to the high occurrence of localized infusion site discomfort, the method of administration was changed in Phase II to co-infusion with approximately 10 mL of autologous blood, which, to date, has elicited neither pain nor toxicity. In over 130 patients treated to date, with the possible exception of one serious adverse event, rectal bleeding, which the Investigator questionably attributed to RRx-001, despite the presence of preexisting hemostatic abnormalities including an increased prothrombin time/international normalized ratio from liver metastases, none have experienced any RRx-001-related systemic toxicities.

3.2. Phase I study

The first-in-man phase I trial (NCT01359982), which evaluated the safety and PK of RRx-001, enrolled 25 patients with widespread histologically confirmed and heavily pretreated cancers, represented by 11 colorectal cancers, four head and neck, two Non-small cell lung cancer (NSCLC) three pancreatic, one cholangiocarcinoma, one ovarian, one melanoma, one brain, and one liver, over six dose levels ranging from 10 mg/m² to 83 mg/m² administered intravenously once or twice weekly. In spite of the local infusion pain, no dose-limiting toxicities were observed in any cohort, and therefore the maximum tolerated dose was not reached. Likewise, no relevant treatment-related changes in laboratory values were observed across any of the dose levels. The most frequently reported adverse effect, irrespective of causality and grade, was pain on infusion, observed in 84% of patients, mostly grades 1 and 2, which immediately resolved following cessation of RRx-001 with no identified long-term safety consequences. The other most frequently reported adverse effects included swelling/edema (32%) in the arm (site of infusion) and vein hardening (28%) [4].

In terms of antitumor activity, a total of 21 patients were evaluated for response by RECIST v 1.1, assessed every 8 weeks. One partial response (1/21, 5%), 14 stable disease
≥8 weeks (14/21, 67%), and six progressive disease (6/21, 29%) were observed. The median overall survival among all patients was 8.2 months, with a median follow-up time of 4.2 months. Following RRx-001 administration, four subjects were successfully rechallenged with previously effective but now refractory therapies, indicative of resensitization. The median overall survival was approximately 18 months (median follow-up time was approximately 13 months) in these four patients, counting from the first dose of RRx-001 administration (Figure 2) [4,15,17].

3.3. Phase II studies

Three main Phase II studies are currently ongoing:

1. QUADRUPLE THREAT (NCT02489903) in refractory/resistant small cell lung cancer, Epidermal Growth Factor Receptor (EGFR) non-small cell lung cancer, high-grade neuroendocrine, and resistant/refractory gynecologic cancers including ovarian and malignant mixed Mullerian tumor;
2. BRAINSTORM (NCT02215512) in brain metastases (RRx-001 in combination with whole brain radiotherapy (WBRT) and temozolomide); and
3. ROCKET (NCT02096354) in colorectal cancer (RRx-001 until progression followed by reintroduction of second-line irinotecan) vs. regorafenib.

The QUADRUPLE THREAT trial conducted in lung, neuroendocrine, and ovarian cancers involves RRx-001 administration until progression followed by reintroduction of first-line platinum doublets (Figure 3(a)). A chemotherapy-free interval (CFI) has been inserted for patients with stable disease or better after completion of the platinum doublets to restore chemosensitivity; the assumption is that, when treatment is removed, the cancer cells will ‘switch’ back to a sensitive phenotype, potentially rendering the tumor susceptible to subsequent therapy; to date, the CFI has been longer than 3 months in four of nine evaluable patients (44%). The trial is ongoing; however, early results have demonstrated resensitization in the form of durable partial responses in six of nine evaluable patients (71%). These partial responses have been accompanied histologically by dense immune infiltrates, fibrosis, cytoplasmic vacuolations (foamy changes) due to macrocytic ingestion of RRx-001 red blood cells, presence of transforming growth factor-beta (TGF-β), and extensive tumor cell necrosis (Figures 3(b) and 4(a)). As a macrophage chemoattractant, TGF-β is a potential biomarker to predict RRx-001 activity along with other chemokines that induce monocyte chemotaxis such as monocyte chemoattractant protein-1, macrophage inflammatory protein-1α, and epidermal growth factor/colony stimulating factor 1.

In the BRAINSTORM trial, patients receive RRx-001 during treatment of brain metastases with whole brain radiotherapy and temozolomide (TMZ) followed by maintenance treatment with RRx-001 and TMZ. The trial is ongoing; however, three of six evaluable patients (50%) with melanoma brain metastases have already demonstrated partial responses (Figure 4(b)).

The clinical trial in colorectal cancer acronymed ‘ROCKET’ has demonstrated numerous instances of resensitization to irinotecan-based therapy in histologically proven mCRC patients with confirmed refractory status after once-weekly treatment with RRx-001 until progression. To date, a significant proportion of RRx-001 patients restarted irinotecan after completion of the first, priming phase of the study, with demonstrated tumor resensitization. Figure 4(c) shows resensitization as determined by a decrease in CEA levels on treatment with irinotecan after exposure to RRx-001 in a representative number of patients. At the first interim analysis, a survival advantage of approximately 2 months in favor of the
RRx-001 arm was observed. Table 2 shows the status of RRx-001 in various clinical trials.

4. Mechanism of therapeutic actions by RRx-001

4.1. RRx-001 covalently binds to the cysteine 93 residue of the Hb β-chain, leading to profound changes in the red blood cell redox status

RRx-001 acts as a ‘ligand’, which binds to, or ‘fits’, a site on hemoglobin, the beta-93 cysteine hemoglobin ‘receptor’, leading to a conformational change in the hemoglobin molecule that initiates a chain reaction mechanism of oxidation inside the red blood cell (Figure 5(a)). This over-oxidation event is the first ‘domino’ to set off the downstream anticancer activity of RRx-001. Preclinical data have demonstrated that RRx-001 incubation with red blood cells ex-vivo results in excessive levels of peroxide [18], which breaks down hemoglobin, releasing the iron [19] and heme from the protein to generate a runaway cascade of damaging free radicals that also leads to membrane scrambling with translocation of the phospholipid, phosphatidylserine (PS), to the erythrocyte surface as well as expression of other molecules such as CD36, CD47, CD71, and ICAM-1 that promote adhesion to the tumor vasculature.

4.2. Tumor microvascular occlusion and Trojan Horse-like RBC phagocytosis by tumor-associated macrophages, leading to their M1 repolarization

The morphological and rheological consequences of the disturbed erythrocyte environment are exacerbated under conditions of profound hypoxia and low blood flow. These modified red blood cells act as nanosized anticancer bioagents: their tendency to aggregate is based on an altered deformability as well as the binding of PS, CD36, CD47, CD71, and ICAM-1, the ‘Velcro’ of the red blood cell membrane, to cognate receptors on the tumor vasculature, which contributes to persistent or recurrent tumor microvascular clogging and stasis [19]. This microvascular occlusion leads to further increase in acidosis and hypoxia, conditions which are required for the hemoglobin-mediated reduction of nitrite to nitric oxide, thereby making RRx-001 a hypoxia-activated
The bound RRx-001-modified RBCs, abundant in oxidized lipids, iron, and heme, are phagocytized by TAM leading to their repolarization from M2-like to M1-like. As a result, the RRx-001-bound red blood cell resembles the ‘Trojan Horse’ of Greek mythology, bearing abundant free radicals in the form of iron, heme, and oxidized lipids, which leads to repolarization of the TAMs in the hypoxic tumor environment (Figure 5(c)).

Supporting experimental evidence for selective tumor nitro-oxidation includes stimulation of inducible nitric oxide in vivo [22], as well as oxidation of tumor thiols detected by enhanced MRI [23]. These data suggest that through the intermediary of the TAM RRx-001 modifies the intracellular tumor environment to a more pro-oxidant phenotype, and any subsequent processes such as epigenetic enzymes, for example, DNA methyltransferases and histone deacetylases (HDACs) that are redox-sensitive may be altered, attenuated, or inhibited.

Tumor cells constitutively express elevated levels of reactive oxygen species (ROS) [24] compared to their nonmalignant counterparts. In addition, the products of the chemical interaction of nitric oxide (NO), a multifunctional free radical, anti-cancer agent that induces its own hypoxia [3,21] (Figure 5(b–d)).

The bound RRx-001-modified RBCs, abundant in oxidized lipids, iron, and heme, are phagocytized by TAM [19,20], leading to their repolarization from M2-like to M1-like. As a result, the RRx-001-bound red blood cell resembles the ‘Trojan Horse’ of Greek mythology, bearing abundant free radicals in the form of iron, heme, and oxidized lipids, which leads to repolarization of the TAMs in the hypoxic tumor environment (Figure 5(c)).

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Tumor cells constitutively express elevated levels of reactive oxygen species (ROS) [24] compared to their nonmalignant counterparts. In addition, the products of the chemical interaction of nitric oxide (NO), a multifunctional free radical,
with ROS are highly toxic oxidants (e.g. such as peroxynitrite (ONOO\(^-\)) and nitrogen dioxide (NO\(_2\)) [25], collectively called reactive nitrogen species. These reactive oxygen and nitrogen species oxidize and damage macromolecules such as carbohydrates, lipids, proteins, and nucleic acids [26]. Normal tissue is spared because the overproduction of reactive oxygen/nitrogen species occurs selectively within the tumor, which contributes to the lack of systemic toxicity.

4.3. Lymphocytic infiltration of the tumor

TAMs isolated from mice bearing B16 melanoma xenografts after treatment with RRx-001 display a predominantly M1 macrophage phenotype, compared to vehicle control. In addition, the supernatant of RRx-001-incubated RBCs switched the TAM phenotype from M2 to M1 [19]. It is hypothesized that this switch occurs as a result of the catabolism of the lipid-rich RRx-001-modified red blood cell, since oxidized low-density lipoprotein is known to activate macrophages through the toll-like receptor 4 [27]. These M1 repolarized TAMs, which express proinflammatory cytokines such as tumor necrosis factor alpha, interleukin-6, and interferon gamma, stimulate the adaptive arm of the immune system as demonstrated by the infiltration of CD8\(^+\) T cells on patient biopsies.

4.4. Vascular ‘normalization’ and increased tumor blood flow

The tumor microvasculature is highly aberrant and circuitous. RRx-001-mediated vascular normalization is thought primarily to be due to repolarization of TAMs; this repolarization leads to an inhibition of VEGF and MMP-9, two critically important mediators of TAM angiogenic potential; a reduction in VEGF and MMP-9 has been observed in immunohistochemically stained patient biopsies. In addition, because RRx-001-modified red blood cells bind to the hypoxic endothelium [28], inducing a microvascular ‘traffic jam’, blood flow is shunted or redistributed through the remaining and more normally functioning (i.e. less leaky, disordered and hypoxic) capillary system, akin to a vascular normalization effect, with a net gain in overall perfusion (Figure 5(c)). Evidence for this concept has been generated preclinically through correlation of contrast agent flow and immunohistochemical results.

RRx-001-treated U87 xenograft (glioma) specimens analyzed immunohistochemically with anti-CD-31, a marker for...
vasculature, overexpressed in the neovascular tumor bed, and a fluorescent carbocyanine dye, DIOC7, that stains cells immediately adjacent to blood vessels and, thus, outlines perfused tumor vasculature, demonstrated increased vessel perfusion [29]. In a separate study of syngeneic SCCVII tumors, quantitative measurements of the flow of an ultrasound microbubble contrast agent demonstrated a significant enhancement of tumor perfusion following treatment with three different doses (3, 6, 12 mg/kg) of RRx-001 compared to control mice, reaching a peak at 6 h after treatment, and returning to baseline within 72 h [1].

In a third study, immunodeficient (SCID) mice bearing P22 sarcoma tumors and instrumented with dorsal window chambers received red blood cells incubated with either RRx-001 or vehicle control on day 4 and 7 post-tumor implantation. The results, shown in Figure 5(d), demonstrated that the red blood cells tended to ‘logjam’ or occlude the tumor vasculature, presumably resulting in blood flow alterations due to vessel pruning or the ‘cutting off’ of vessels and normalization [19].

These vascular normalization results have been recapitulated in a Phase I/II clinical trial with DCE-MRI assessment of brain metastases, demonstrating decreased vessel leakage and improved perfusion after treatment with RRx-001. Vascular normalization is also thought to be secondary to reduced VEGF and MMP-9, two critically important mediators of TAM angiogenic potential that has been observed in immunohistochemically stained patient biopsies.

4.5. Epigenetic modulation

In response to RRx-001-induced reactive oxygen and nitrogen oxide species, DNA methyltransferases and histone deacetylases that contain redox-sensitive active site cysteines are inhibited, thereby modulating protein function and activity. Pre-clinically, RRx-001 has demonstrated multiple epigenetic modulation effects, concordantly inhibiting HDACs, DNA methyltransferases [30,31], and lysine demethylases and relieving gene silencing mechanisms. Since drug resistance is thought to be a polygenic phenotype caused by reversible changes in the expression of multiple genes, the inhibition of epigenetic enzymes that maintain transcriptional silence has the potential to reactivate genes known to impact chemosensitivity.

SCCVII and MM.1S tumor cells treated with RRx-001 exhibit genome wide re-expression of genes such as the tumor suppressor, p53 [22]; in this way, RRx-001 may ‘re-program’ the genome and increase the efficacy of co-therapies such as radiation and conventional chemotherapy.

4.6. Immuno-, radio-, and chemosensitization of tumor tissue

Preliminary evidence indicates that the underlying mechanism responsible for RRx-001-mediated sensitization in addition to increased blood delivery and tumor oxygenation is M1 macrophage elimination of CSCs, which are known to contribute to chemoresistance and recurrent disease. Secondarily, because mechanisms of chemoresistance are multifactorial, epigenetic modulation and inhibition of drug efflux transporters also seem to be involved [1]. Phase I [17] and Phase II [32] clinical trials have demonstrated that RRx-001 sensitizes tumors to different chemotherapeutic agents including irinotecan, cisplatin/carboplatin, docetaxel, paclitaxel, nab-paclitaxel, etoposide, bevacizumab, and 5-FU. Chemosensitization also has been observed preclinically in cancers of the brain and in multiple myeloma [31], while immunosensitization (potentiation of checkpoint inhibitor activity) has occurred in a myeloma J558L model [1]. Similar studies have revealed that RRx-001 sensitizes glioma and epidermal cancer to gamma radiation without increased toxicity. Emerging clinical data in the BRAINSTORM trial in brain metastases support these findings.

4.7. Protection of normal tissue from radiation chemotherapy damage

Paradoxically, for a radiosensitizer, RRx-001 has demonstrated protection of the gastrointestinal tract from radiotherapy-induced toxicity in a murine study. The protective effects of RRx-001 on normal tissue may be mediated through the induction of the Nrf2-regulated antioxidant cell response, which neutralizes free radical damage [2]. In addition to radioprotective properties, RRx-001 is also associated with chemoprotection. The effect of pretreatment with RRx-001 was studied on cisplatin-induced nephroto-, myelo-, and genotoxicity in BALB/c mice. The mice were divided into three groups: (1) no treatment, (2) vehicle and cisplatin only, and (3) RRx-001 and cisplatin. RRx-001 treatment (5 mg/kg every other day for three days) was initiated 3 days prior to cisplatin administration. Renal dysfunction was evaluated biochemically by measuring the concentration of blood urea nitrogen and serum creatinine. Genotoxicity was evaluated by metaphase spreads from whole bone marrow cells. Myelotoxicity was evaluated with measurement of serum hemoglobin, leukocyte, and platelet concentrations.

Cisplatin significantly elevated the levels of blood urea nitrogen, serum creatinine, and the kidney to body weight ratio, but pretreatment with RRx-001 significantly attenuated the cisplatin-induced nephrotoxicity. After administration of cisplatin, the frequency of chromosomal abnormalities distinctly increased. However, in mice pretreated with RRx-001, consistent improvement in chromosome spreading with lower frequencies of broken metaphases was obtained compared with mice injected with only cisplatin, as seen in the Figure 6(a).

These results suggest that RRx-001 has a protective effect against cisplatin-induced nephro-, myelo-, and geno-toxicity in normal tissue but not tumors. Similar to the putative mechanism of radioprotection, activation of the Nrf2 pathway is thought to be responsible for these chemoprotective effects.

5. Discussion

The developmental trajectory of RRx-001 is an example of phenotypic discovery [33] ‘old school’ R&D, a ‘throwback’ to an earlier empiric era before—omics and targeted ‘me too’ therapies became oncologic bywords and reconfigured the clinical lay of the land. Prior to the advent of personalized
medicine and precision oncology, novelty of mechanism and structure, evidence of in vivo activity and lack of toxicity at clinically relevant doses, all defining characteristics of RRx-001, were more important go/no go criteria than in-depth and a priori knowledge of the molecular target. The first question to be asked (and answered) in the development process of RRx-001 was ‘does it work?’ not ‘how does it work?’

Ironically, given its broad spectrum of activity in the absence of systemic side effects, RRx-001 is more highly targeted than, for example, the kinase inhibitors, which carry a label of ‘precision medicine’ and are referred to as ‘targeted’, but because binding can occur in all tissues of the body, can result in off-target (non-tumor specific) toxicities that affect virtually every organ system in the body [34]. The ironic legacy of the molecularly targeted agents is that they are no less toxic or more ‘precise’ than the standard chemotherapy they were designed to replace, and while targeted therapies with broad activity, such as multitarget inhibitors, possess both activity and toxicity in equal measure, highly selective kinase inhibitors often have activity tuned out.

In many cases, these kinase-targeted therapies may, at times, result in tumor resistance [35], not only to Tyrosine-kinase inhibitors (TKIs) but also to several other unrelated agents. By contrast, RRx-001 generally primes tumor cells to respond (and respond) to concomitant or subsequent radiotherapy, chemotherapy, and immunotherapy. The implication of RRx-001 pretreatment on increased susceptibility is prolonged survival, since multiple retreatments with RRx-001 may repeatedly radiosensitize tumor cells and potentially render a once fatal disease chronic, similar to the story of triple therapy and HIV infection.

6. Conclusion

In summary, based on all the preclinical and clinical data, RRx-001 not only appears to have single agent anticancer activity in the absence of systemic toxicity but also (a) increases blood flow to tumors, which facilitates delivery of exogenously administered therapeutics, thereby increasing their cytotoxic effects, (b) synergizes with radiation, and (c) ‘prosensitizes’ tumors, a hybrid word, referring to chemosensitization of tumors as well as chemoprotection and radioprotection of normal tissue. Subjects with advanced heavily pretreated and drug-resistant disease may particularly benefit from the pro-vascular, prosensitizing, immunologic, and epigenetically mediated priming effects of RRx-001. Whether or not RRx-001 ultimately fulfills these lofty expectations and sets a new standard for ‘targeted agents’ in oncology will be determined in several radiation, chemotherapy, and immunotherapy combination Phase II and Phase III clinical trials. In a best-of-all-possible-world’s scenario, treatment with RRx-001 will act as a ‘reset button’ on tumor resistance, to permit the repeated re-use of previously effective first- and second-line therapies, hopefully adding time (years rather than mere months), but not toxicity, to the lives of cancer patients.

7. Expert opinion

As a small molecule derived from an unlikely source, the aerospace industry, RRx-001 possesses unique biological (as well as structural) properties and mechanisms that set it apart from other anticancer agents. As a macrophage stimulating and
prosensitizing agent with clinical and preclinical evidence of anti-cancer immune activity, RRx-001 has the potential to synergize with chemotherapy, radiotherapy, and immunotherapy in the neoadjuvant, concomitant, and adjuvant context without any apparent additional toxicity. Moreover, unlike the cytoprotectant amifostine (WR-2721), which may inadvertently afford protection to the tumor as well as normal tissues, RRx-001 appears to minimize damage to normal tissues and critical organs while simultaneously enhancing the radiochemosensitivity of cancer cells.

Although immune checkpoint inhibitors such as programmed cell death 1, programmed cell death ligand-1, and cytotoxic T-lymphocyte-associated protein 4 have garnered substantial attention and interest due to the demonstration of robust and durable responses, the fact remains that 70–80% of patients with susceptible tumor types such as NSCLC, melanoma, and bladder do not benefit from them. Hence, the integration of RRx-001 with immunotherapy is a conceptually promising strategy to augment systemic antitumor immune responses in these patients.

Moreover, since future treatment options are limited once resistance (inevitably) emerges, any new FDA-approved salvage therapy, especially a non-toxic one like RRx-001 that may reverse the refractory phenotype and even possibly protect normal tissue against the cytotoxic effects of radiation and chemotherapy, is likely to be prescribed not only on an on-label basis but also off-label in an attempt to extend survival without adversely affecting quality of life. The next step for RRx-001 is Phase III trials in lung cancer (SCLC, NSCLC), neuroendocrine and/or melanoma brain metastases to confirm efficacy, hopefully resulting in FDA approval in at least one of these indications within 3–5 years.

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**Declaration of interest**

S. Knox is a founder of Epicent Rx, B. Oronsky and J. Scicinski are employees of Epicent Rx, N. Abrouk is an employee of Innovexe and A. Oronsky is an employee of InterWest Partners. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

**References**

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.


• This reference is the first and in some ways the most comprehensive data paper on RRx-001, which is referred to herein by its chemical acronym, ABDN AZ.


•• This reference is of considerable interest because the boosting of Nrf2 activity with RRx-001 is thought to explain its radioprotective and chemoprotective effects on normal tissues.


• The Lancet Oncology paper summarizes the safety profile of RRx-001 in Phase 1 as well as introducing its potential to re-sensitize to subsequent chemotherapies.


• This review, which describes how and why RRx-001 quickly reacts with sulfur nucleophiles, partly explains the lack of systemic toxicity since thiol-bound RRx-001 is unavailable to combine directly with nucleic material (e.g. DNA).


• The manuscript, which deconvolutes the sequential metabolism and disposition of RRx-001, integrates data from *in vitro* models, animal studies, and human clinical studies.


• Of interest because it highlights the potential for versatility of RRx-001 in other non-oncologic indications and because it provides proof-of-concept for pharmacologic activity across the blood brain barrier.

11. Fens MHAM, Larkin SK, Morris CR, et al. NO or no NO, increased reduction of nitrite to nitric oxide by modified red blood cells. ASH Annu Meet Abstr. 2011;118:2125.


• Since macrophages are an integral part of the antitumor mechanism of RRx-001, this paper serves as a good and clinically-focused review of the role of macrophages in cancer.


This data paper unpacks the complex interplay of processes and mechanisms that result in the anti-cancer activity of RRx-001 specifically in multiple myeloma.


