Multi-Chemotherapeutic Schedules Containing the pan-FGFR Inhibitor ARQ 087 are Safe and Show Antitumor Activity in Different Xenograft Models

Abstract

ARQ 087 is a multi-tyrosine kinase inhibitor with potent activity against the FGFR receptor family, currently in Phase I clinical studies for the treatment of advanced solid tumors. The compound has a very safe profile and induces tumor regressions in FGFR-driven models. The feasibility of combining ARQ 087 with chemotherapy was investigated in FGFR deregulated human xenografts. Nude mice were transplanted subcutaneously with H1581, and when tumor masses reached 150 mg, were randomized to receive vehicle, ARQ 087, paclitaxel, carboplatin as single agents or in combination. Similar experimental conditions were applied in nude mice bearing SNU16 and MFE296 xenografts, with the inclusion of capecitabine in the former xenograft model. In the different xenograft models, the drugs given as single agents ranged from very active to partially active. The double combinations were more active than the single ones, but the triple combinations were the most active. In particular, the combination of ARQ 087 + paclitaxel + carboplatin in H1581 bearing mice was able to induce tumor regression in all the mice, with 6/8 mice tumor free at day 140 after tumor transplant. Of note, no toxic deaths nor premature stopping or delaying of drug administration were observed. The data herein reported demonstrated the feasibility of using xenografts models for poli-chemotherapeutic trials mimicking the best standard of care in treatment of specific tumor type and that ARQ 087, a new pan-FGFR inhibitor, can be safely combined with standard cytotoxic chemotherapeutic drugs with apparently no sign of cumulative toxicity and an associated increased antitumor effect.

Introduction

Historically, treatment of cancer includes a combination of surgery, chemotherapy, radiotherapy and immunotherapy with the goal to improve the therapeutic index, resulting from the summing of anticancer effects and the non-overlapping side effects of the different treatment modalities [1,2]. Indeed these poli-chemotherapeutic schedules proved important not only for the long-term control of many adult and most pediatric hematological malignancies, but also have been shown to lower the emergence of resistant clones. The increased understanding of tumor biology has led to the identification of tumor drivers (i.e. KRAS, epidermal growth factor receptor-EGFR-, fibroblast growth factor receptor-FGFR-) whose inhibition with targeted agents has showed to have antitumor activity and lead to their approvals for the treatment of human cancer [3,4]. An important part of the development of new targeted agents is the testing of the agent in combination with standard care (radio and chemotherapy) bearing in mind the above discussed considerations, i.e. their use in combinatory schedules to increase antitumor activity and to possibly delay the onset of resistance [5,6]. Improved patient overall survival has indeed been
demonstrated that when standard chemotherapy was associated with trastuzumab in breast cancer [7], with bevacizumab in colon cancer [8] and in non-small cell lung cancer [9] and when cetuximab was combined with radiation in head and neck tumors [10]. Recently, the possibility to combine targeted agents seems to represent a valid therapeutic approach and assumed to be necessary to optimize the success of treatments [11].

ARQ 087 is a novel, ATP competitive, small molecule, multi-kinase inhibitor, which showed in vitro and in vivo activity, mainly against FGFR deregulated systems. Biochemically, ARQ 087 inhibits FGFR1, FGFR2, mutant FGFR2 (N549H), FGFR3, and FGFR4 kinases, with IC₅₀ values in the low nanomolar range in biochemical assays. Cell proliferation studies demonstrated that ARQ 087 has anti-proliferative activity in different cell lines with higher activity in whose cells driven by FGFR dysregulation, including amplifications, fusions, and mutations. The potent drug cytotoxic activity translated in ARQ 087 in vivo antitumor activity in different tumor type including deregulated FGFR systems [12]. The drug is currently in phase I clinical trial, where positive signs of activity, including one partial response and two durable responses, have been seen in a subset of patients with FGFR2 fusion positive intrahepatic cholangiocarcinoma [13].

We here report the feasibility of combining ARQ 087 with chemotherapeutic agents in different xenografts models.

Materials and Methods

Drugs

For in vivo studies, drugs were prepared as follows and given at doses and schedules specified in Results. ARQ 087, kindly provided by ArQule, was dissolved in 10% dimethylacetamide, 10% cremophor, 10% propylene glycol, 50% acetate buffer at pH 5 and given orally. Paclitaxel, provided by ChemieTek, was dissolved in cremophor/absolute ethanol (1:1), subsequently diluted in saline solution and injected intravenously. Carboplatin, provided by Sigma, was dissolved in saline solution and injected intravenously. Capecitabine, provided by Sigma, was dissolved in saline solution and given orally. All the treatments were done as 10 ml/kg.

Xenograft Models

For antitumoral activities on H1581, MFE296, SNU16 models, seven- to twelve-week-old female NCr-nu/nu mice were obtained from Envigo, Udine, Italy. Mice were maintained under specific pathogen-free conditions, housed in isolated ventilated cages, and handled using aseptic procedures. Procedures involving animals and their care were conducted in conformance with institutional guidelines in compliance with national and international laws and policies.

Xenograft models were obtained from H1581 lung cancer, MFE296 and SNU16 gastric cancer. The characteristics of the xenografts used are summarized in Supplementary Table 1. Fragments of H1581, MFE296 and SNU16 were implanted s.c. into the right flank region of athymic mice. Mice were randomized when the average tumor size was 150 mg to receive single drug treatments and combinations as specified in the Results. Each group consisted of eight animals. Mice body weight was recorded three times a week. Tumor growth was measured three times a week with a Vernier caliper, and the tumor weights (mg = mm³) were calculated as follows: (length [mm] × width [mm]²)/2. Efficacy was expressed as best treated/control tumor weight mean × 100 (T/C%) (day).

According to the NCI criteria, values of 42 or lower indicate that the treatment can be considered active [14]. Mice were sacrificed when tumor masses reached 10% of body weight.

Results

The pharmacological characterization of ARQ 087 has recently been reported [12]. The drug has been shown to have in vitro cytotoxic activity and in vivo antitumor activity, especially in FGFR deregulated systems. The drug has been reported to be active and safe when administrated daily for 10–15 days at a dose range of 50–150 mg/kg. The molecular characterization of the xenografts tested is reported in Supplementary Table 1.

The feasibility of combining ARQ 087 with different cytotoxic drugs was tested in lung (H1581), gastric (SNU16) and endometrial (MFE296) xenografts and the choice of the anticancer agents to be combined with ARQ 087 was based on the fact that the selected drugs are component of the multi chemotherapy schedules clinically used in the treatment of these neoplasms. All the cytotoxic drugs were given at doses that have been previously reported as active and safe [15–18] while ARQ 087 was given daily for 15 days at the dose of 75 mg/kg, schedule recently reported to have in vivo antitumor effect [12].

Lung Cancer Model (H1581)

Nude mice were subcutaneously transplanted with H1581 xenograft and when tumor masses reached 150 mg mice were randomized to receive ARQ 087, paclitaxel, carboplatin and their combinations as specified in Figure 1 legend. All the treatments were well tolerated as demonstrated by the lack of specific side effects and a mice body loss lower than 20% (Figure 1A). In this xenograft model (Figure 2A, Table 1), as single agents, ARQ 087 showed moderate antitumor activity, carboplatin was inactive and paclitaxel was very active. All the paclitaxel including schedules induced tumor regressions; however mice treated with a combination of paclitaxel and ARQ 087 or with paclitaxel, carboplatin and ARQ 087 experienced a higher degree of tumor regression and a longer time to progression than single agents and the combination paclitaxel and carboplatin (Figure 2A). Interestingly, 5 out of 8 mice treated with the triple combination were tumor-free at day 140 (Figure 2A and Table 1), when mice were to be sacrificed.

Endometrial Cancer Model (MFE296)

In MFE296 xenograft model, ARQ 087 and carboplatin as single agents were almost inactive, while paclitaxel was active (Figure 2B, Table 1). Interestingly enough, tumor stabilizations, with some tumor regressions, were observed only in mice treated with ARQ 087 and paclitaxel (but not in the paclitaxel/carboplatin combination) and mice treated with the triple combination. Again, all the treatments were well tolerated (Figure 1B).

Gastric Cancer Model (SNU16)

In SNU16 xenograft model, ARQ 087 showed moderate antitumor activity, while carboplatin and capecitabine were inactive (Figure 2C, Table 1). All the treatments were well tolerated (Figure 1C). While ARQ 087 double combinations were more active than ARQ 087 single agent both in term of tumor growth (Figure 2C) and corresponding T/C% values (Table 1); the triple combination showed the best antitumor activity, with some tumor regressions observed and 1/9 tumor-free animal at the end of experiment (Table 1).

Discussion

Cancer therapy is a multimodal therapy based on the fact that combination of anticancer agents with different mechanisms of action
have higher antitumor activity than single agent treatment. In addition, it is well established that the use of drug combinations could help in circumventing tumor resistance. Multi-chemotherapeutic schedules in cancer combine agents with known activity, different mechanism of action, possibly different mechanism of resistance, and minimally overlapping spectra of toxicity at their optimal doses. These considerations, well proven for standard cytotoxic therapy and/or radiotherapy, also apply when combining targeted agents [5]. In fact, in recent years FDA and EMA have both approved targeted agents in combination with chemotherapy based on the increased overall survival and/or progression free survival in tumor patients with the combinations [5,7–9]. Considering that combination therapy is the standard in the treatment of some cancers, the development of new anticancer agents (both cytotoxic and targeted agents) should include the evaluation of potential combinations with chemotherapeutic and targeted agents as early as reasonable during the development cycle.

ARQ 087 is a novel small molecule pan-FGFR inhibitor, able to inhibit FGFR family members at nanomolar concentrations, and other important kinase targets such as KDR and PDGR at higher concentrations [12]. The in vitro data suggested that the drug is particularly active in FGFR deregulated systems (mutations/amplification/fusions). These data were corroborated in an in vivo setting, in which the drug has clearly shown a good antitumor activity and a safe profile in different xenograft models [19,20]. Of note, in the ongoing phase I clinical trial positive signs of activity, one partial response and two durable responses, have been observed in a subset of patients with FGFR2 fusion positive intrahepatic cholangiocarcinoma [13].

The feasibility of the inclusion of ARQ 087 in polichemotherapeutic schedules and in combination with targeted agents was herein addressed. We selected xenografts models harboring known FGFR deregulated pathways. In our experimental setting we tried to recapitulate the clinical situation adding ARQ 087 on the top of the best standard of care for each tumor type considered. Paclitaxel,
carboplatin and capecitabine could be safely administered with ARQ 087; indeed all the triple combinations were well tolerated suggesting that the drug can be safely incorporated in the clinical used multi-chemotherapeutic schedules with reasonable lack of fatal overlapping toxicities. In addition, in the H1581 lung xenograft model a clear improvement of antitumor activity was observed with 5 out of 8 mice tumor-free, and potentially cured, at 140 days after tumor transplantation. In the other two experimental systems, an additive effect of the combination was observed. We have not yet addressed the molecular basis of the synergistic activity of the combination of ARQ 087, paclitaxel and carboplatin. Available data suggest that single ARQ 087 treatment caused a G1 block of the cell cycle and this is in line with the lack of apoptosis induction, suggesting that its main effect is cytostatic. It is well demonstrated that paclitaxel is a microtubule interfering agent and carboplatin is an alkylating agent causing respectively a G2-M block [21] and S-G2 block of the cell cycle [22,23] and possibly able to induce apoptosis [24]. When these combined treatments were tested in the same cell lines as continuous treatment, no synergistic effects were observed (data not shown). The causes of these apparently contrasting results are likely due to the fact that treatments modality are very different between the in vitro and in vivo setting; i.e. in vitro it was a continuous co-treatment, while in vivo the three drugs have a specific pharmacokinetic profiles with different tumor drug accumulation. In addition, we cannot rule out that both ARQ 087 and paclitaxel have antiangiogenic properties [25] contributing to the synergistic effect observed in vivo, but not in vitro. Experiments are ongoing to specifically address this point.

The data herein reported demonstrated the feasibility of using xenografts models for poli-chemotherapeutic trials mimicking the best standard of care in treatment of specific tumor type and that ARQ 087, a new pan-FGFR inhibitor, can be safely combined with both standard cytotoxic chemotherapeutic drugs and targeted agents, with apparently no sign of cumulative toxicity and increased antitumor effect.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.tranon.2016.12.003.

Conflict of Interest Statement
None declared.

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Table 1. Antitumor Activity of ARQ 087 as Single Agent or in Combination with Chemotherapy in H1581, MFE296 and SNU16 Xenograft Systems

| Treatment schedule | H1581 T/C% (day) | MFE296 T/C% (day) | SNU16 T/C% (day) |
|--------------------|-----------------|-----------------|-----------------|
|                    | No. of tumor-free animals (day) | No. of tumor-free animals (day) | No. of tumor-free animals (day) |
| ARQ087 + CAPECITABINE | 64.9 (43) | 0/8 | 32 (28) |
| ARQ087 + CARBOPLATIN | 9.2 (36) | 0/8 | 36 (28) |
| ARQ087 + CAPECITABINE | 30 (30) | 0/8 | 64.9 (43) |
| ARQ087 + TAXOL | 73.6 (28) | 0/8 | 64.9 (43) |
| ARQ087 | 44.3 (30) | 0/8 | 64.9 (43) |
| CAPECITABINE | 52.6 (37) | 0/9 | 32 (28) |
| TAXOL | 27.4 (36) | 0/8 | 64.9 (43) |
| CARBOPLATIN | 64.9 (43) | 0/8 | 64.9 (43) |
| ARQ087 + CAPECITABINE + CARBOPLATIN | 9.6 (29) | 0/9 | 32 (28) |
| ARQ087 + TAXOL + CARBOPLATIN | 9.6 (29) | 0/9 | 32 (28) |
| ARQ087 + CAPECITABINE | 9.6 (29) | 0/9 | 32 (28) |
| ARQ087 + CARBOPLATIN | 9.6 (29) | 0/9 | 32 (28) |
| ARQ087 + TAXOL | 9.6 (29) | 0/9 | 32 (28) |
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| TAXOL | 27.4 (36) | 0/8 | 64.9 (43) |
| CARBOPLATIN | 64.9 (43) | 0/8 | 64.9 (43) |
| ARQ087 + CAPECITABINE + CARBOPLATIN | 9.6 (29) | 0/9 | 32 (28) |
| ARQ087 + TAXOL + CARBOPLATIN | 9.6 (29) | 0/9 | 32 (28) |
| ARQ087 + CAPECITABINE | 9.6 (29) | 0/9 | 32 (28) |
| ARQ087 + CARBOPLATIN | 9.6 (29) | 0/9 | 32 (28) |
| ARQ087 + TAXOL | 9.6 (29) | 0/9 | 32 (28) |
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| ARQ087 + CAPECITABINE | 9.6 (29) | 0/9 | 32 (28) |
| ARQ087 + CARBOPLATIN | 9.6 (29) | 0/9 | 32 (28) |
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| ARQ087 + CAPECITABINE | 9.6 (29) | 0/9 | 32 (28) |
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| ARQ087 + CAPECITABINE | 9.6 (29) | 0/9 | 32 (28) |
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| ARQ087 + CAPECITABINE + CARBOPLATIN | 9.6 (29) | 0/9 | 32 (28) |
| ARQ087 + TAXOL + CARBOPLATIN | 9.6 (29) | 0/9 | 32 (28) |
| ARQ087 + CAPECITABINE | 9.6 (29) | 0/9 | 32 (28) |
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| ARQ087 + TAXOL + CARBOPLATIN | 9.6 (29) | 0/9 | 32 (28) |