Published OnlineFirst August 24, 2015; DOI: 10.1158/1535-7163.MCT-15-0253

Small Molecule Therapeutics

Molecular Cancer Therapeutics

# Targeting G-Quadruplex DNA Structures by EMICORON Has a Strong Antitumor Efficacy against Advanced Models of Human Colon Cancer

Manuela Porru[1], Simona Artuso[1], Erica Salvati[1], Armandodoriano Bianco[2], Marco Franceschin[2], Maria Grazia Diodoro[3], Daniela Passeri[4], Augusto Orlandi[4], Francesco Savorani[5], Maurizio D'Incalci[6], Annamaria Biroccio[1], and Carlo Leonetti[1]

## Abstract

We previously identified EMICORON as a novel G-quadruplex (G4) ligand showing high selectivity for G4 structures over the duplex DNA, causing telomere damage and inhibition of cell proliferation in transformed and tumor cells. Here, we evaluated the antitumoral effect of EMICORON on advanced models of human colon cancer that could adequately predict human clinical outcomes. Our results showed that EMICORON was well tolerated in mice, as no adverse effects were reported, and a low ratio of sensitivity across human and mouse bone marrow cells was observed, indicating a good potential for reaching similar blood levels in humans. Moreover, EMICORON showed a marked therapeutic efficacy, as it inhibited the growth of patient-derived xenografts (PDX) and orthotopic colon cancer and strongly reduced the dissemination of tumor cells to lymph nodes, intestine, stomach, and liver. Finally, activation of DNA damage and impairment of proliferation and angiogenesis are proved to be key determinants of EMICORON antitumoral activity. Altogether, our results, performed on advanced experimental models of human colon cancer that bridge the translational gap between preclinical and clinical studies, demonstrated that EMICORON had an unprecedented antitumor activity warranting further studies of EMICORON-based combination treatments. *Mol Cancer Ther; 14(11); 2541–51. ©2015 AACR.*

## Introduction

G-quadruplex (G4) ligands are small molecules able to bind to and stabilize G4 structures widely described at the telomeric ends of chromosomes (1–6). Interest in the more general therapeutic significance of G4 has expanded during the past decade including G4 structures in the promoters of a wide range of genes important in cell signaling, recognized as hallmarks of cancer. More interestingly, G4 DNA structures have been now visualized in human cells, corroborating the concept of G4 ligands as therapeutically susceptible hotspots (6, 7).

Over the past 2 decades, several G4-interacting small molecules, used to act as G4 stabilizers, have been developed with the aim of identifying potential anticancer therapeutics. In this regard, growing evidence shows that G4 ligands selectively impair the growth of cancer cells without affecting the viability of normal cells pointing out these molecules as possible drug candidates for future clinical application (8, 9). This evidence gains further support by the marked antitumoral activity showed by some of those compounds (BRACO-19, RHPS4, and telomestatin) in different *in vivo* models both as single agents and in combination with conventional or targeted anticancer agents (10–15). However, none of the other G4 ligands developed so far has made it through the drug discovery pipeline due to poor drug-like properties and/or selectivity profile (16, 17). This has strongly encouraged the researchers to make further efforts in the identification of G4-binding agents as potential anticancer drugs. In this context, significant results were obtained by the employment of tetrasubstituted naphthalene diimide compounds as quadruplex-binding ligands, in inhibiting cancer cell growth both *in vitro* and *in vivo* (18–20). Our group has recently designed a new molecule, namely, EMICORON (Fig. 1A), having one piperidinyl group bound to the perylene bay area, sufficient to guarantee a good selectivity, and an extended aromatic core able to increase the stacking interactions with the ending tetrad of the G4. The subsequent profiling of its biologic properties demonstrated that this compound is outstandingly potent in inducing selective DNA damage to telomeres of cancer cells versus normal cells, endowing with efficient antiproliferative effect on several tumor cell lines at low micromolar concentrations (21). Moreover, in agreement with its ability to target the G4 sequence identified in the promoter of VEGFR-2 (22), EMICORON can bind to G4 structures throughout the genome (Fig. 1B), highlighting this drug as a novel

[1]Experimental Chemotherapy Laboratory, Regina Elena National Cancer Institute, Rome, Italy. [2]Department of Chemistry, University of Rome "Sapienza", Rome, Italy. [3]Department of Pathology, Regina Elena National Cancer Institute, Rome, Italy. [4]Department of Biopathology and Image Diagnostics, Anatomic Pathology Institute, University of Rome "Tor Vergata", Rome, Italy. [5]Department of Food Science, Faculty of Science, University of Copenhagen, Copenhagen, Denmark. [6]Department of Oncology, Pharmacological Research Institute "Mario Negri", Milan, Italy.

**Note:** Supplementary data for this article are available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org/).

M. Porru and S. Artuso contributed equally to this article.

**Corresponding Authors:** Carlo Leonetti, Regina Elena National Cancer Institute, via Elio Chianesi, 53, Rome 00144, Italy. Phone: 39-0652662534; Fax: 39-0652662592; E-mail: leonetti@ifo.it; and Annamaria Biroccio, biroccio@ifo.it

**doi:** 10.1158/1535-7163.MCT-15-0253

Published OnlineFirst August 24, 2015; DOI: 10.1158/1535-7163.MCT-15-0253



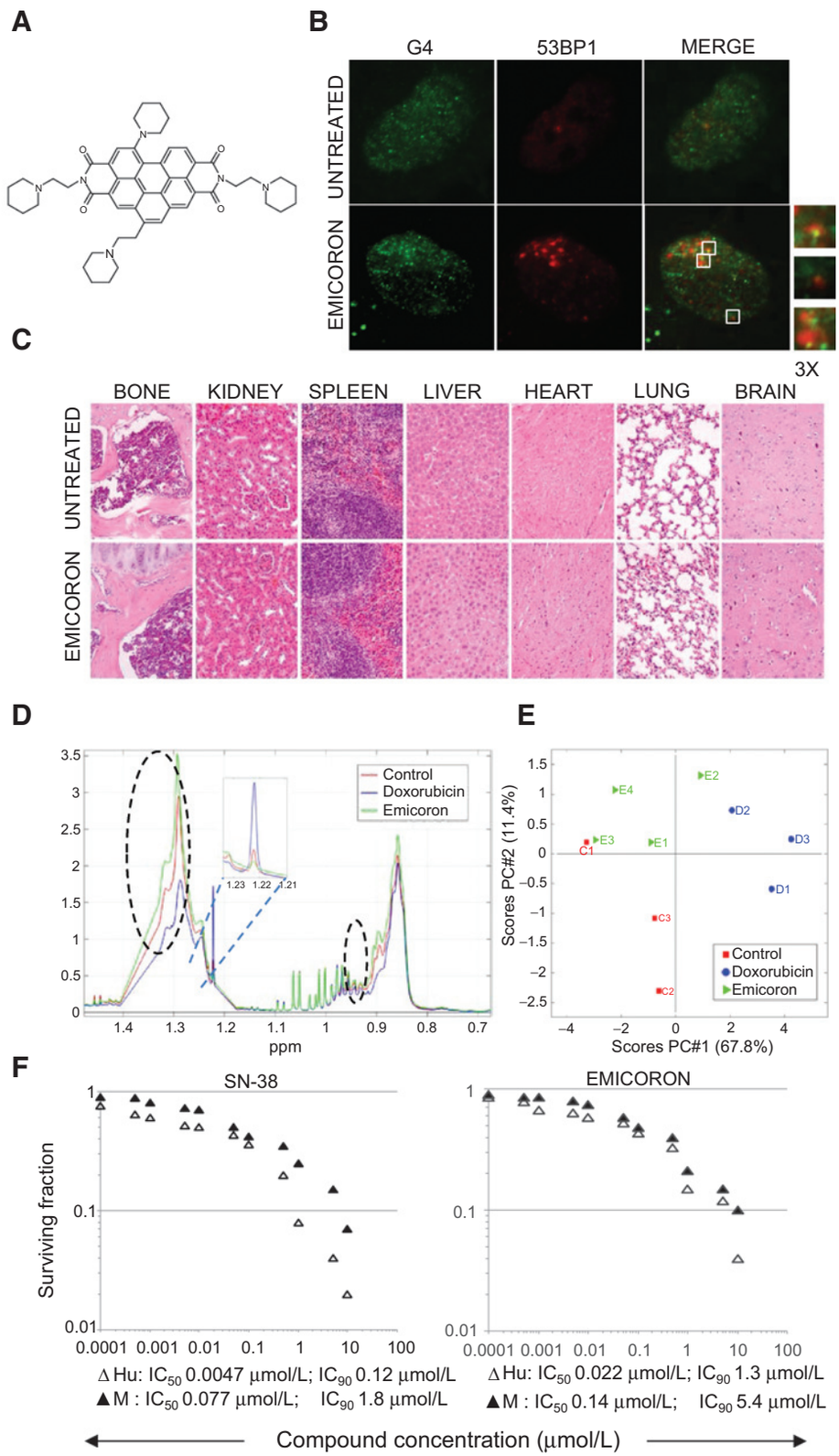


**Figure 1.**
Toxicologic profile of EMICORON. A, structural formula of EMICORON. B, targeting of G4 structures upon EMICORON treatment. BJ EHLT immortalized human fibroblasts were exposed to 0.5 μmol/L EMICORON for 24 hours and then fixed and co-immunostained with anti-G4 and anti-53BP1 antibodies to detect DNA damage foci. Representative images at ×100 magnification are reported. Enlargements show colocalization between DNA damage foci, induced by EMICORON, and G4 structure. C, histologic analysis of tissue from untreated or EMICORON-treated mice. Twenty-four hours after the end of treatment, mice were euthanized and tissues placed in 10% buffered formalin for 24 hours, dehydrated, and embedded in paraffin. Serial 4-μm-thick sections were stained with H&E and analyzed by optical microscope. Original magnification, ×200. D, average spectra of blood from mice untreated or treated with doxorubicin and EMICORN, in the NMR lipid and lipoprotein spectral area (CPMG experiments). The regions in which the differences are more pronounced are circled or zoomed in. It is mostly the lipid and lipoprotein profiles that are affected, especially for what it concerns the doxorubicin group. E, PCA scores plot of the CPMG spectral data collected for each mouse (2 outliers, C4 and D4, are removed). PC#1, which explains almost 70% of the total variance, shows that the control and the EMICORON groups are behaving similarly, whereas the doxorubicin one tends to be different. F, concentration–response curves of SN-38 and EMICORON in bone marrow CFU-GM assays. Human (△) or mouse (▲) marrow cells were continuously exposed for 15 or 13 days, respectively, to drugs at half-log intervals covering 7 logs and colonies, defined as clusters containing 30 or more cells, were counted and $IC_{50}$ and $IC_{90}$ were determined from concentration–response curves.

Published OnlineFirst August 24, 2015; DOI: 10.1158/1535-7163.MCT-15-0253



multitargeting G4-interacting agents warrant further studies aimed at assessing its antitumoral activity in preclinical models.

Despite many antineoplastic compounds showed favorable tumor responses in preclinical studies, more than 95% of these therapeutics failed to confirm efficacy in clinical trials and many factors are responsible for this high failure rate (23, 24). In particular, the different sensitivity of murine and human bone marrow progenitor cells to cytotoxic drugs is critical for the translation of new compounds from preclinical studies to clinical application. In fact, only compounds with similar bone marrow progenitor sensitivity across species may have better potential of reaching the same blood level in patients as in mice (25). To this purpose, it has been suggested that the use of bone marrow colony-forming unit, granulocyte-macrophage (CFU-GM) assay comparing the toxic effects on bone marrow from animals and humans could help us identify drugs with good potential for achieving a therapeutic blood level in patients (26, 27). Moreover, it is a common opinion that a further major limitation to translate cancer research from bench to bedside is the lack of predictive cancer models. In particular, while ectopic human tumor xenografts from established cell lines could be useful for a first assessment of efficacy of new anticancer compounds, the use of orthotopic models helps us validate compounds against tumor growth in the site of origin, progression, and metastatic dissemination (28, 29). In this context, genetically engineered mouse models (GEMM) have become a useful system that, by recapitulating diseases that derive from genetic changes such as cancer, could predict human responses in the clinic better than xenografts from established cancer cell lines and could also be a platform for discovering predictive biomarkers (30). Finally, the recent introduction of patient-derived xenografts (PDX), obtained by implanting tumor fragments from patients to immunocompromised mice, thus preserving genomic integrity and tumor heterogeneity observed in patients, could increase the robustness of drug discovery studies (31, 32). On the basis of this background, the aim of this study was to evaluate the antitumor efficacy of the new G4 ligand EMICORON on a spectrum of advanced preclinical models of colon cancer that recapitulate some key points to be considered for the translatability of experimental findings, including maintenance of the biologic characteristics of human cancer in terms of tumor architecture and mutational status, growth in the origin tissue in the context of a species-matched tumor stroma microenvironment, and intact immune system.

## Materials and Methods

### Drugs

*N*,*N*-Bis[2-(1-piperidino)-ethyl]-1-(1-piperidinyl)-6-[2-(1-piperidino)-ethyl]-benzo[ghi] perylene-3,4:9,10-tetracarboxylic diimide [EMICORON; molecular formula: $C_{52}H_{59}N_6O_4.4HCl$; molecular weight = 977, estimated $pK_a$ (piperidine) = 11] was synthesized as previously described (21) and used as a salt, specifically hydrochloride, with a good solubility in water. 7-Ethyl-10-hydroxycamptothecin (SN-38, Alexis) and doxorubicin (Ebewe) were also used.

### Animals and ethics statement

Six- to 8-week-old male mice CD-1 nude (*nu*/*nu*), C57BL/6, BALB/c (Charles River Laboratories) and NOD/SCID (Harlan Laboratories), were used. All animal procedures were approved by the ethics committee of the Regina Elena National Cancer Institute (CE/534/12) and were in compliance with the national and international directives (D.L. March 4, 2014, no. 26; directive 2010/63/EU of the European Parliament and of the council; Guide for the Care and Use of Laboratory Animals, U.S. National Research Council, 2011).

### Human cell lines and PDXs

A90 colon epithelial tumor cell line from GEMMs was obtained on October 2013 by E.S. Martin (Dana-Farber Cancer Institute, Boston, MA). Primary colon cancer cells were obtained by the mechanical disaggregation of a colon adenocarcinoma specimen surgically treated at the Regina Elena National Cancer Institute (Rome, Italy) on January 30, 2013. Both cell lines were not authenticated. Cells were maintained as monolayer culture in RPMI-1640 medium supplemented with 10% FCS, L-glutamine, and antibiotics. PDXs were obtained by the implant in mice of tumor fragment from a metastatic lymph node of a poorly differentiated colon carcinoma of a patient at Regina Elena National Cancer Institute. Briefly, surgical specimens, not required for histopathologic analysis, were placed in medium supplemented with antibiotics, diced into 15 to 20 $mm^3$ pieces, coated in Matrigel, and implanted into NOD/SCID mice by a small incision and subcutaneous pocket made in one side of the lower back (33). After mass formation in mice (passage 0), tumors were passed in 4 mice (passage 1) and afterward expanded in 20 mice (passage 2).

### CFU-GM assay

According to the protocol described by Kurtzberg and colleagues (25), mouse marrow was collected from the long bones (femur and tibia) of healthy Balb/c mice. Bones were flushed with sterile RPMI-1640 supplemented with 5% FBS by using a 27-G syringe. The resulting cell suspension ($\sim 1.5 \times 10^7$ cells per mouse) was collected in a 50-mL conical-bottom tube and kept in wet ice. Human bone marrow cells (StemCell Technologies) were prepared according to the vendor protocol. For mouse CFU-GM assays, mouse bone marrow cells were cultured in MethoCult (StemCell Technologies), whereas MethoCult GF medium was used for human CFU-GM assays. A total of $4 \times 10^4$ mouse cells per plate and $8 \times 10^4$ human cells per plate were set up in duplicate 100-$cm^2$ culture dishes and continuously exposed to EMICORON or SN-38 at a half-log interval concentration covering 7 logs, for 13 days for mouse bone marrow and 15 days for human bone marrow. Three independent CFU-GM colony formation experiments were performed. Colonies were defined as clusters containing 30 or more cells.

### *In vivo* toxicological studies

To determine the maximum tolerated dose (MTD) of EMICORON, this compound was freshly dissolved in saline solution (0.9%) and given *per os* by oral gavage in healthy mice at doses from 400 to 800 mg/kg in a single bolus. Then, a chronic administration of EMICORON at 1:10 of the MTD for 15 days was evaluated to identify the best scheduling of treatment. Toxicity was assessed on the basis of drug-related moribund state, death, body weight loss, and other signs including mouse behavior and movement. At the end of treatment, mice were euthanized and major organs excised for histologic analysis. Each individual experiment included 3 mice.

Published OnlineFirst August 24, 2015; DOI: 10.1158/1535-7163.MCT-15-0253



### Metabolomics study

BALB/c mice were treated with EMICORON given *per os* at 15 mg/kg/d per 15 consecutive days or intraperitoneally with doxorubicin at 10 mg/kg in a single treatment, and 24 hours after the end of treatments, blood was collected into heparinized tubes, plasma prepared and stored at −80°C. Each of the 12 plasma samples, representing the control group (4) and the 2 treatment groups (4 EMI + 4 doxorubicin), was thawed on ice and the 5-mm NMR tubes prepared and analyzed using a standardized and highly automated procedure on the basis of the guidelines introduced by Beckonert (34). NMR analysis was performed with a Bruker Avance III 600 spectrometer (Bruker Biospin Gmbh) operating at 600.13 MHz for protons, equipped with a double tuned cryo-probe (TCI). Spectra were acquired at 310 K using both the Carr–Purcell–Meiboom–Gill (CPMG) experiment and the NOESY-presat one (34). To correct for unwanted signal shifts, the entire dataset was globally aligned with respect to the α-D-glucose signal around 5.25 ppm using the icoshift algorithm (35). In total, 24 spectra were acquired and processed using MATLAB 2013b (The MathWorks Inc.).

### Bioluminescence imaging analysis

Primary colon cancer cells and A90 cells were stably transfected with the PGL2 vector containing the firefly luciferase gene under control of the SV promoter (Promega). Human xenografts were imaged using the IVIS imaging system 200 series (Caliper Life Sciences). Briefly, mice were anesthetized with a combination of tiletamine—zolazepam (telazol; Virbac) and xylazine (xylazine/Rompun BAYER) given intramuscularly at 2 mg/kg. Then mice were injected intraperitoneally with 150 mg/kg D-luciferin (Caliper Life Sciences) and imaged in the supine position 10 to 15 minutes after luciferin injection. Data were acquired and analyzed using the Living Image Software version 3.0 (Caliper Life Sciences).

### Antitumor activity

EMICORON was given *per os* by gavage at 15 mg/kg for 15 consecutive days. In particular, luminescent primary colon cancer cells were injected ($10^6$ cells/mouse) in the spleen of anesthetized nude mice, and after 30 minutes, the spleen was removed. Twelve days postinjection, when tumor dissemination was evident, treatment started. Real-time tumor dissemination in mice treated with EMICORON or untreated was monitored using the IVIS imaging system. Thirty-two days after injection, the mice were sacrificed, liver and gastrointestinal organs harvested, and imaged. Each group included 6 mice.

A90 luminescent (A90-LUC) GEMM murine colon cancer cells were injected in anesthetized C57BL/6 mice, with a 27-gauge needle at $10^5$ cells per mouse in 50 μL (50% Matrigel and 50% medium-free serum), into the rectal mucosa approximately 1 to 2 mm beyond the anal canal, which minimizes the chance of establishing anal tumors. Mice were treated with EMICORON, starting from day 7 after cell injection, when the presence of tumor in the rectum was observed. Imaging was performed at baseline (day 7) before the administration of compound, and at days 15 and 23 (2 days after the end of treatment), when mice were euthanized for ethical reasons and tumor excised for histologic analysis. Each group included 6 mice.

A cohort of 20 PDXs at passage 2 were allowed to grow to about 300 to 350 $mm^3$ before initiation of treatment. Ten mice were treated with EMICORON and 10 mice were used as control (untreated). Antitumor efficacy of treatment was assessed by the following endpoints: (i) percent tumor volume inhibition; (ii) tumor growth delay, evaluated as $T - C$, where $T$ and $C$ are the median times for treated and control tumors, respectively, to achieve equivalent size; (iii) stable disease, defined as the maintenance for at least 3 weeks of the same tumor volume as the start of treatment; and (iv) increase of mice survival. The animals were euthanized for ethical reasons when tumors reached a mean of 2.5 to 3.0 $cm^3$ in volume or when they became moribund during the observation period (the time of euthanization was recorded as the time of death).

### Histologic and immunohistochemical studies

Paraffin-embedded 4-μm thick sections from major organs or tumors were stained with hematoxylin and eosin (H&E) and analyzed by optical microscope. The tumor and necrotic areas and the mitotic index were calculated by morphometric methods (36). Immunohistochemical analyses were performed by using the following antibodies: rat monoclonal anti-CD31 (1:100, BD Pharmingen); rabbit monoclonal anti-Ki-67 (1:200, Novus Biologicals); mouse monoclonal anti-phospho-histone H2AX (γH2AX, 1:150, Millipore); anti-cytokeratin 7 (CK7, clone OV-TL 12/30, Dako); anti-cytokeratin 20 (CK20 clone Ks20.8, Dako); and anti-CDX2 (CDX2, clone CDX2-88, Biocare Medical). The number of tumor vessels per $mm^2$ on zinc-fixed CD31-stained sections and the proliferation as percentage of Ki-67–positive nuclei were evaluated in at least 10 randomly selected fields. Necrotic areas were excluded. The number of γH2AX-positive cells per $mm^2$ was counted in eight high-power fields per section.

### Immunofluorescence

Treated and untreated cells were fixed in 2% formaldehyde and permeabilized in 0.25% Triton X-100. Then, cells were incubated with the following primary antibodies for 2 hours at room temperature: anti-G4 antibody (1H6 monoclonal antibody, P. Lansdorp, ERIBA, University of Groningen, Groningen, the Netherlands) and anti-53BP1 antibody (Novus Biological Inc.) and then washed in PBS and incubated with the following secondary antibodies: TRITC-conjugated goat anti-rabbit and FITC-conjugated goat anti-mouse (Jackson ImmunoResearch). Fluorescent signals were recorded by using a Leica DMIRE2 microscope equipped with a Leica DFC 350FX camera and elaborated by a Leica FW4000 deconvolution software (Leica).

### Statistical analysis

The Student *t* test (unpaired, two-tailed) was used for comparing statistical differences. Survival curves of mice were generated by Kaplan–Maier product-limit estimate, and statistical differences between the various groups were evaluated by log-rank analysis with Yates correction (software Primer of Biostatistics, McGraw-Hill). Differences were considered statistically significant when $P < 0.05$.

## Results

### EMICORON exhibits a favorable preclinical toxicologic profile

We initially assessed the MTD of EMICORON by treating mice with high doses of the drug, given by oral gavage in a single

Published OnlineFirst August 24, 2015; DOI: 10.1158/1535-7163.MCT-15-0253



**Table 1.** Identification of MTD of EMICORON in healthy mice

| Treatment[a] | Toxic deaths | Body weight loss, % | Tachypnea | Dysentery | Dehydration |
|---|---|---|---|---|---|
| (a) 800 mg/kg | 0/3 | 22 | Yes | Yes | Yes |
| (b) 600 mg/kg | 0/3 | 0 | No | Yes | Yes |
| (c) 400 mg/kg | 0/3 | 0 | No | No | No |
| (d) 20 mg/kg/d × 15d | 0/3 | 30 | No | No | No |
| (e) 15 mg/kg/d × 15d | 0/3 | 8 | No | No | No |

[a]Mice were treated with EMICORON given by oral gavage in a single administration (groups a–c) or fractionated for 15 days (groups d and e).

administration (400, 600, or 800 mg/kg/mouse). We decided to evaluate this route of administration, as oral administration of chemotherapeutic agents to patients with cancer in a pill form has significant advantage over other routes of administration, avoiding complications associated with intravenous or intraperitoneal dosing and not requiring hospitalization or special care. As reported in Table 1, the highest dose administered (800 mg/kg) was very toxic as a body weight reduction of 22% was recorded. In addition, immediately after treatment mice elicited a transient tachypnea, which passed off in few minutes. Moreover, during the first week after treatment, dysentery and dehydration were reported in mice. These symptoms were not more observed in the next weeks and mice progressively recovered the initial body weight. After the injection of the dose of 600 mg/kg, a reduction of about 10% of body weight was reported accompanied by dysentery observed for a couple of days in 1 of 3 treated mice. Finally, the dose of 400 mg/kg was well tolerated and animals showed good conditions, as no body weight loss or other symptoms were observed, thus identifying this one as the MTD. On the basis of our previous observations showing that to obtain antitumoral efficacy, G4-interactive compounds need to be administered for several days, we fractionated the MTD of EMICORON in more injections. Our results (Table 1) demonstrated that while the treatment at 20 mg/kg/d for 15 consecutive days produced a marked body weight loss, the reduction of the dose at 15 mg/kg/d was free of adverse effects. The good tolerability of EMICORON was confirmed by histologic analysis of the major organs of mice treated with this scheduling. In fact, brain, lung, heart, liver, spleen, kidney, and femoral bone tissue did not display microscopic difference compared with the mice treated with the vehicle demonstrating that this scheduling did not produce adverse effects (Fig. 1C).

A pilot metabolomic study using NMR analysis was also conducted in which the potentiality of this new and promising approach was tested to assess whether the metabolic differences induced in the mice blood by EMICORON could support the classical toxicologic approaches. Figure 1D neatly shows that the average metabolome of the mice treated with EMICORON resembles that of the control group more than the metabolome of the mice treated with doxorubicin. Indeed, the whole lipid profile metabolome is significantly suppressed in the mice treated with doxorubicin, very likely due to the toxicity of this molecule, whereas the same is not valid for the mice treated with EMICORON, which show a similar lipid profile with the control group (Fig. 1D and Supplementary Fig. S1A and S1B). Further evidence pointing toward such a result was evidenced by the multivariate data analysis that highlights the latent variables and summarizes and describes the characteristics of each mouse (Fig. 1E and Supplementary Fig. S1C and S1D).

Before moving along the antitumor efficacy studies, we sought to compare the sensitivity of mouse and human bone marrow to EMICORON treatment by using the CFU-GM assay. This because mouse bone marrow is generally less sensitive to many cytotoxic agents than human bone marrow, allowing in preclinical experiments blood levels that cannot be achieved in patients. Therefore, compounds having small or no differential in bone marrow progenitor sensitivity among species may have a better translational potential. With this aim, mice or human bone marrow cells were exposed to different concentration of EMICORON. As control, the sensitivity of mice and human bone marrow CFU-GM to the well-studied Top1 inhibitor SN-38 (the active metabolite of irinotecan) was evaluated. The concentration–response curves are reported in Fig. 1F, and the ratios of the mouse and human bone marrow CFU-GM at $IC_{50}$ and $IC_{90}$ concentrations were calculated. As expected and in agreement with previously published data (25, 26), our results confirm that human bone marrow is more sensitive to SN-38 than the mouse one, as the ratios mouse/human for this camptothecin were about 15-fold. Interestingly, the ratios of mouse/human CFU-GM $IC_{50}$ and $IC_{90}$ were about 5-fold for EMICORON, thus suggesting for this compound a good potential for reaching similar blood levels in humans as in mice.

### EMICORON has a marked antitumoral activity against a colon cancer PDXs

With the aim of assessing the efficacy of EMICORON in preclinical models preserving the biologic characteristics of human colon cancer, in terms of tumor architecture and mutational status, so having a predictive power in the translation into clinical settings (31), experiments were performed on PDXs. Histologic and immunohistochemical analysis showed that PDXs retain morphologic characteristics of the original malignancy (Fig. 2A). Next, we evaluated the antitumoral activity of the drug by treating the mice with EMICORON at 15 mg/kg for 15 consecutive days. This scheduling of drug administration has been chosen on the basis of preliminary data (Supplementary Table SI). Moreover, according to individual mouse variability in terms of tumor appearance, we decided to consider each mouse as one tumor-bearing entity. Treatment started for each mouse when a tumor mass of about 300 to 350 $mm^3$ was evident. As shown in the Fig. 2B, a progressive increase of tumor mass was observed in untreated PDXs, whereas EMICORON-treated mice exhibited a marked reduction of the tumor growth, being all tumors reduced comparing with the untreated ones. Looking at the overall response rates (Table 2), EMICORON produced a significant inhibition of the growth of tumors (64%, $P = 0.000$) and a tumor growth delay of 13 days. Interestingly, in 2 of the 6 mice treated, a stabilization of the disease was observed. Finally, the inhibition of the tumor growth by EMICORON treatment resulted in a 90% increase of mice overall survival with a median survival time of 63 days (49–76) that was significantly longer ($P = 0.000$) compared with 33 days (19–41) observed in untreated group (Table 2 and Fig. 2C).

Immunohistochemical analysis, performed 2 days after the end of treatment in tumors sections from untreated and EMICORON-treated mice, revealed that the strong antitumor efficacy of

Published OnlineFirst August 24, 2015; DOI: 10.1158/1535-7163.MCT-15-0253



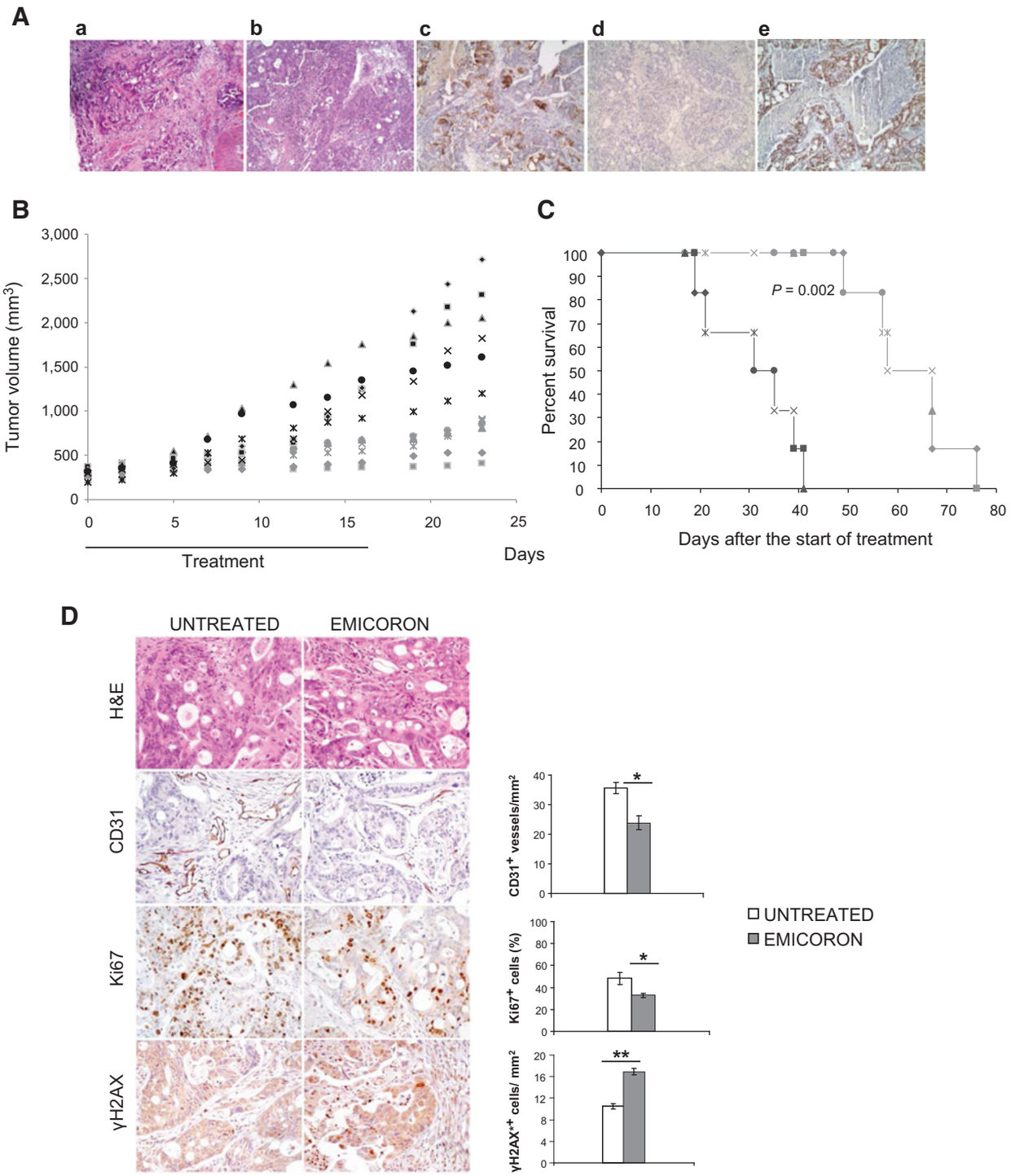


**Figure 2.**
Therapeutic efficacy of EMICORON on PDXs. A, morphologic analysis of the original patient tumor and PDXs. Tumor tissue from a metastatic lymph node of a poorly differentiated colon adenocarcinoma of the patient (a) and from the PDXs (b) were compared after H&E staining. To determine the morphologic stability of the xenografts, IHC analysis of CK-7 (c) and CK-20 (d) as markers of the epithelium and of CDX2 (e), the intestinal transcriptional factor, was performed. Original magnification, ×400. B, tumor growth curves of untreated (black symbols) and EMICORON-treated (gray symbols) PDXs. Each symbol represents the same mouse. Six mice for each group were reported. C, survival curves of untreated (black symbol) or EMICORON-treated (gray symbols); D, representative images of tumor excised from untreated or EMICORON-treated mice, euthanized 2 days after the end of treatment and semiquantitative evaluation of CD31, Ki-67, and γH2AX immunostainings on paraffin xenopatient-derived tumor sections. Four mice for each group have been evaluated. Four-micrometer-thick tumor sections were stained with H&E or with anti-CD31, Ki-67, γH2AX antibodies. Original magnification, ×400. Data are mean ± SD. *, $P < 0.05$; **, $P < 0.01$.

Published OnlineFirst August 24, 2015; DOI: 10.1158/1535-7163.MCT-15-0253



**Table 2.** EMICORON has therapeutic efficacy against PDXs and GEMMs

| Group model[a] | TWI[b], % | $T - C$[c], days | Stable disease/ mice treated[d] | ILS[e], % |
|---|---|---|---|---|
| PDXs | 64 | 13 | 2/6 | 90 |
| A90-LUC GEMM | 80 | n.e. | 3/6 | n.e. |

Abbreviation: n.e., not evaluable.

[a]Mice were treated with EMICORON *per os* at 15 mg/kg/d for 15 consecutive days.

[b]Tumor weight inhibition was calculated at the nadir of the effect. Statistical analysis: PDX treated versus untreated, $P = 0.000$. A90-LUC GEMM treated versus untreated, $P = 0.002$.

[c]Calculated as the median times for treated (*T*) and control (*C*) tumors to reach the same size (1,000 mg). Statistical analysis: PDX treated versus untreated, $P = 0.001$.

[d]Stable disease was defined as the maintenance for at least 2 weeks of the same tumor weight as the start of treatment.

[e]Increase in lifespan. ILS of treated mice was calculated compared their median survival time with those of untreated mice. Statistical analysis: PDX treated versus untreated, $P = 0.000$.

EMICORON resulted from the inhibition of the angiogenesis and the proliferation, in association with the activation of DNA damage (Fig. 2D). Indeed, in treated tumors, the mean number of tumor vessels per $mm^2$ was significantly ($P < 0.05$) reduced to less than 25 compared with the 35 reported in the untreated group. At the same time, the percentage of Ki-67–positive nuclei was significantly ($P < 0.05$) decreased by about 40% in treated tumors compared with the controls. Consistently, after treatment with EMICORON, a significantly ($P < 0.01$) increased mean number of γH2AX-positive nuclei/$mm^2$ was observed in comparison with untreated ones (16 vs. 10).

### EMICORON inhibits the growth of orthotopic colorectal tumors

The antitumoral efficacy of EMICORON was also evaluated in an orthotopic model of colon cancer by injecting A90-LUC GEMM cells in the rectum of mice, and the bioluminescence was used for noninvasive monitoring of the tumor burden in the whole mice. This model combines the *in vivo* drug validation in the tissue of origin of the primary tumor type in the context of a species-matched tumor stroma microenvironment and an intact immune system (30, 37). The quantification of bioluminescence, shown in Fig. 3A, demonstrated the strong efficacy of EMICORON in inhibiting the growth of tumor. In fact, from the analysis performed at day 7 (start of treatment), 16, and 23 (2 days after the end of treatment), it is evident that while untreated animals elicited more than 4-fold increase of luciferin signal, the bioluminescence in mice treated with EMICORON did not significantly increase from the baseline to the end of treatment, thus suggesting the stabilization of the disease. Figure 3B shows representative images of untreated and treated mice. Moreover, the marked difference in tumor mass between untreated and treated mice was evident by the analysis performed on H&E-stained tumor sections obtained from euthanized mice at day 23 after tumor cells injection (Fig. 3C). In particular, from the calculation by morphometric methods, a significant ($P < 0.05$) reduction of tumor area in EMICORON-treated tumor compared with untreated was noticed. Moreover, the very significant ($P < 0.01$) reduction of mitosis seems to be responsible for the strong antitumoral activity of EMICORON in this orthotopic model of colon cancer. Consistent with its ability to target telomeric G4 structures, EMICORON significantly induced the presence of atypical mitotic figures, which are indicative of telomere dysfunction.

### EMICORON reduces metastatic dissemination of luminescent primary cultured human colon cancer cells

Because a major problem in the management of human colon cancer is the treatment of distant metastases, we evaluated the efficacy of EMICORON on the experimental model of a disseminated disease established after intrasplenic injection of primary colon cancer cells. In Fig. 4, it is reported the disease spread in gastrointestinal tract and in liver evaluated at the start of EMICORON treatment (day 12) and at day 32 (5 days after the end of treatment). Histograms demonstrate that while more than a 4-fold increase of photons/sec was observed in untreated group, EMICORON produced the inhibition of the cancer cell dissemination being the luminescence significantly ($P < 0.01$) reduced of about 50% compared with untreated mice. This is evident both for the analysis performed the in whole mice (A and B) and in organs excised after the euthanization of animals (C and D).

## Discussion

A strong discrepancy between preclinical efficacy and clinical response is observed for cancer disease, as only 5% of agents having anticancer activity in preclinical development are licensed, which is much lower than, for example, 20% for cardiovascular disease (23, 24). Several are the reasons for this high attrition rate, including the complexity of the disease and the need for new clinical trial approaches to improve selection of the best dose, as well as the application of a biomarker-driven patient subselection strategy to better identify responsive patients (38). Unquestionably, the preclinical strategies to evaluate novel agents are suboptimal and the identification of appropriate preclinical mouse cancer models remains a major challenge to increase drug development efficiency. We recognize that each different type of animal cancer model has intrinsic advantages and limitations and that no single model can capture all the different aspects of tumor growth characteristics and treatment approaches. Rather, they should be viewed as a portfolio of sophisticated biologic tools that can be used optimally in the drug discovery process to answer specific experimental questions (39, 40). Accordingly, in this article, we have studied the antitumor efficacy of the new G4 compound EMICORON on different preclinical models of colon cancer that may mimic the clinical situation.

A valuable and generally accepted biologic approach to the study of cancer biology and therapeutics is the ectopic (subcutaneously or intramuscularly) implant of tumor cells into syngeneic (genetically identical) or immunocompromised rodents. Despite these utilities, ectopic models possess limited pathophysiologic relevance and clinical predictability due to the nonphysiologic growth location in the host, and the absence of critical stromal and microenvironmental spatial and paracrine interactions with host noncancerous cells and tissues, including endothelial cells, inflammatory cells, and tumor-associated fibroblasts (40, 41). A more accurate preclinical representation of human colon cancer is the orthotopic injection of tumor cells or implant of tumor tissue. In this article, we have performed a transanal injection of colon cancer cells, which does not require invasive abdominal surgery and lead to the growth of a clinically relevant tumor in the rectum of mice. The translational relevance of this model is reinforced by the fact that to generate the orthotopic tumor, we have injected GEMM cells which carry the genetic and phenotypic signatures of the native cancer, reproduce tumor–stromal

Published OnlineFirst August 24, 2015; DOI: 10.1158/1535-7163.MCT-15-0253



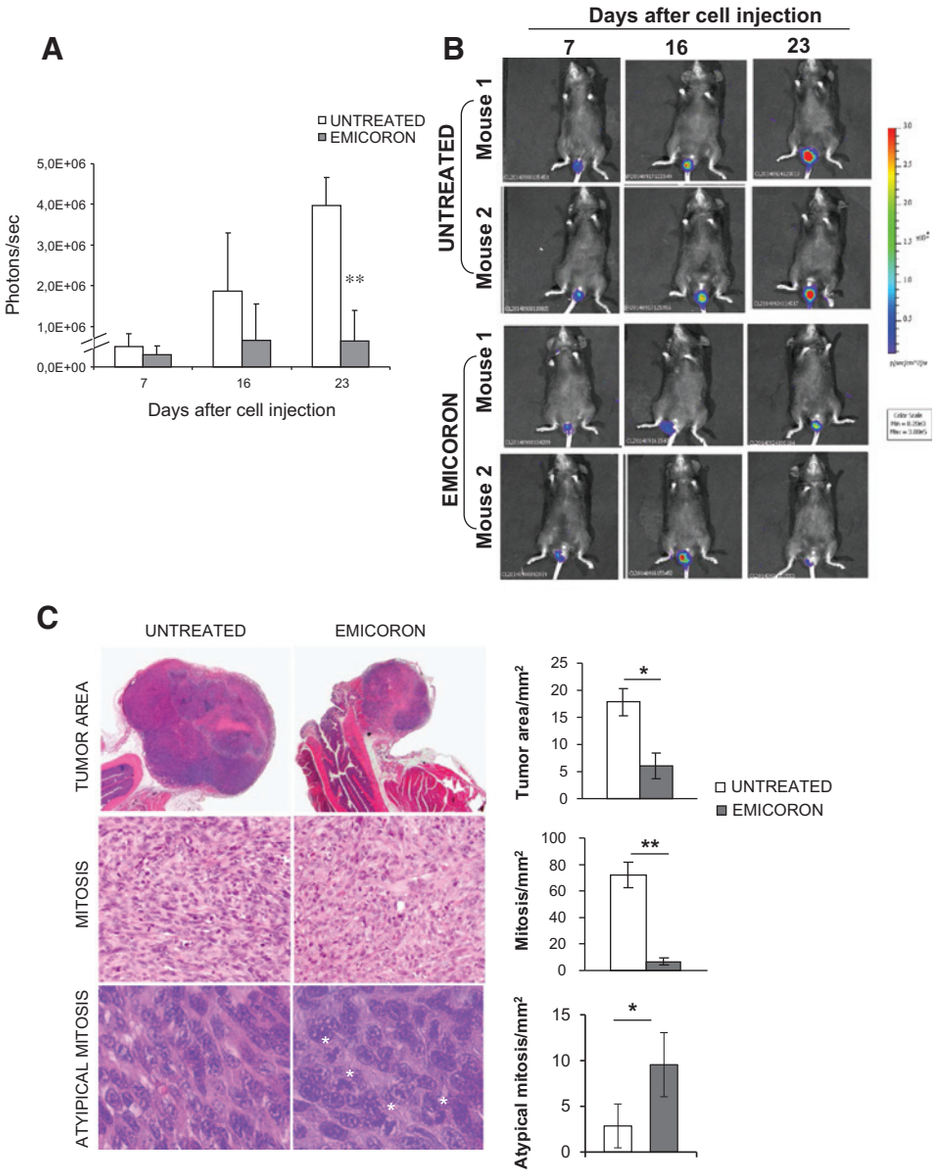


**Figure 3.**
Antitumor activity of EMICORON on orthotopic model of colon cancer. A90-LUC colorectal murine cells were injected in the rectal mucosal surface of mice and EMICORON was administered from days 7 to 21 after tumor cell injection. The real-time tumor growth was monitored by optical imaging. A, histogram reports bioluminescence in tumors from untreated and treated groups. B, representative images of untreated and EMICORON-treated mice. C, two days after the end of treatment (day 23), mice were euthanized, tumors excised, and after staining with H&E, tumor area and mitotic index per $mm^2$ was evaluated. In the same samples, the number of atypical mitosis was scored at ×63 magnification. Representative images of tumor area at ×10 original magnification and mitosis at ×20 and ×63 original magnification. Data are mean ± SD from 6 mice per group.
*, $P < 0.05$; **, $P < 0.01$.

interactions, and may be predictive of eventual therapeutic response in human clinical trials (37). From this point of view, the marked reduction of tumor in the mice rectum after EMICORON treatment, evidenced by the reduction of bioluminescence signal and tumor area, as shown by histologic examination, seems to be very promising for translational purposes.

Because the leading cause of deaths of patients with colon cancer is the metastatic dissemination and in particular the liver colonization, it is imperative to assess the therapeutic benefits of a new compound on experimental models of metastatic disease. The experiments we performed in a well-established model of colon dissemination disease, by intrasplenic injection of tumor cells (42, 43), demonstrated that EMICORON treatment is able to inhibit the spreading of metastasis to both gastrointestinal organs and liver. The most recent class of preclinical models is PDXs, where tumor fragments from patients are directly implanted in immunodeficient mice and then passed directly from mouse to mouse *in vivo*. A relevant characteristic of this model is that PDXs well recapitulate the heterogeneity of tumors in patients and maintain molecular, genetic, and histologic heterogeneity typical of tumors of origin through serial passaging in mice. On the contrary, cell lines, even when propagated *in vivo*, are derived from cancer cells that have adapted to growth outside a natural tumor microenvironment, resulting in genetic changes that are distinct from the genetic stress imposed on tumors in patients (44). Notwithstanding some limitations due to the drift of stromal components from human to mouse, PDXs represent a great challenge for oncology drug development, if viewed as complementary to other preclinical models (45). In this study, we have demonstrated that PDXs showed fidelity to the original tumor, as they maintain the morphologic features of a poorly differentiated colon adenocarcinoma and conserved the same KRAS mutation in the region of exon 2 of codon 12, observed in tumor of patient. Interestingly, while patient was insensitive to chemotherapy,

Published OnlineFirst August 24, 2015; DOI: 10.1158/1535-7163.MCT-15-0253



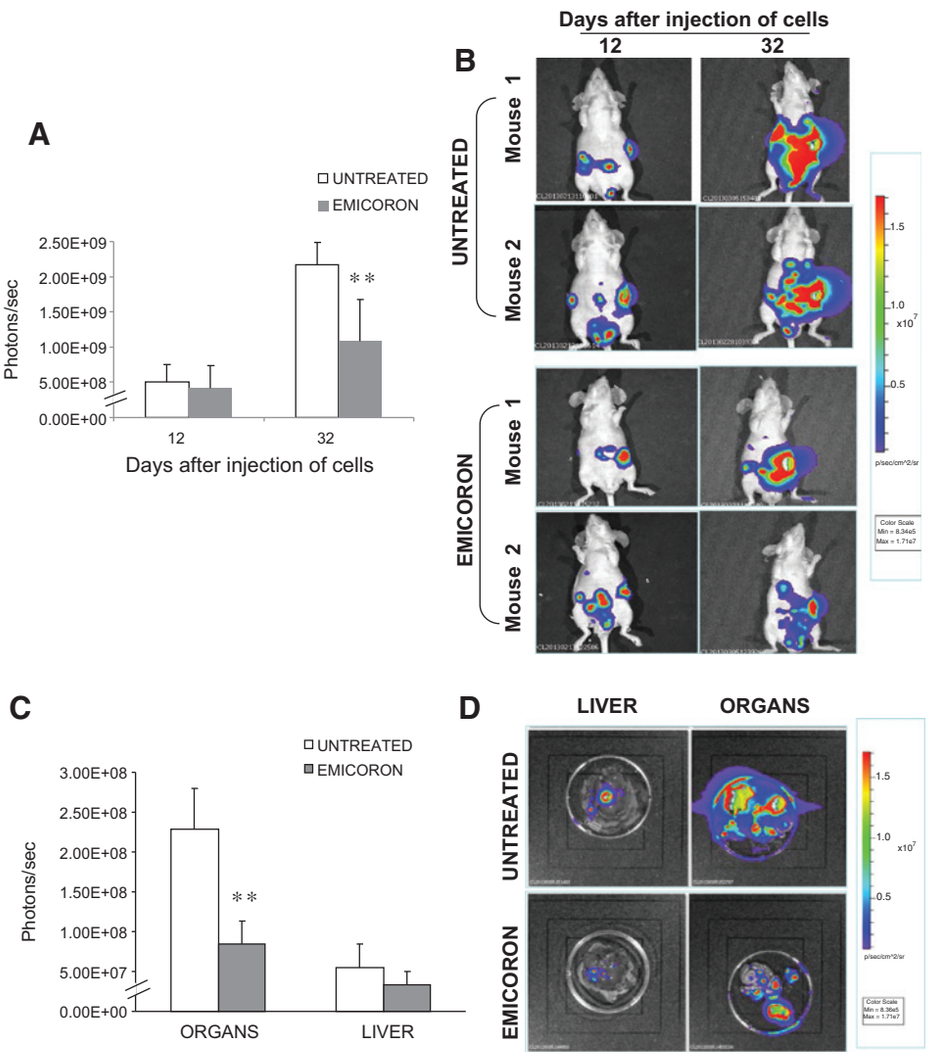


**Figure 4.**
Effect of EMICORON treatment on a model of colon cancer disseminated disease. Luminescent primary colon cancer cells were injected in the spleen of immunosuppressed mice ($n = 12$). Real-time tumor dissemination was monitored by the imaging system. A, histograms report bioluminescence in mice untreated (6 mice) or treated with EMICORON (6 mice) at day 12 after tumor cell injection (start of treatment) and at day 32 after tumor cell injection. B, representative images of mice. C, at day 32, mice were sacrificed; organs were harvested and acquired using the imaging system. D, representative images of liver and gastrointestinal organs. Bars, mean values ± SD. **, $P < 0.01$.

EMICORON treatment was very effective against PDXs as resulted by the strong inhibition of tumor growth, the stabilization of the disease in 2 of 6 mice treated and the significant improvement of the overall survival of mice.

Notably, antitumor efficacy observed at the dose of 15 mg/kg was consistent with the safety of EMICORON treatment, as no body weight loss or toxic deaths were observed in mice and histologic analysis on major organs confirmed the lack of adverse effects. Moreover, a further evidence of the good tolerability of EMICORON is highlighted by the metabolomic analysis. Indeed, even EMICORON has an impact on the mouse metabolome that can be further studied with a more systematic metabolomic approach, it results rather clear that the effects caused by EMICORON are less pronounced than those observed with doxorubicin, making this drug a good candidate for low toxicity. However, given the cardiovascular off-target effects (interaction with the human recombinant β2 adrenergic receptor and M1, M2 and M3 muscarinic receptors, together with a high inhibition of the hERG) observed with the G4 ligand RHPS4 compromising the acceptability of this drug as a clinical candidate (16), data on this are essential before moving EMICORON into clinic. In this context, pharmacokinetic data, including time course analysis of drug absorption, distribution, metabolism, and excretion will be relevant, especially as the compound does not have the appearance of a conventional drug and will permit us to improve the therapeutic efficacy of the drug and have more information that can justify the very high apparent MTD dose.

A key point in drug development from preclinical studies to clinical application is the use of optimal dose, but a frequent observation is that murine bone marrow progenitors are less sensitive to compounds compared with human ones. This means that the drug blood levels achieved in mice producing preclinical efficacy cannot be reached in patients, thus resulting in the failure of clinical trials. On the basis of these observations, it has been suggested that compounds with little differential in bone marrow sensitivity across species may have greater potential for reaching similar blood levels in patients as in mice (25). So, on the basis of the CFU-GM assay developed by Pessina and colleagues (46) and validated in several studies (26, 47, 48), we demonstrated that the mouse bone marrow is only about 5-fold less sensitive than human bone marrow, thus suggesting that EMICORON could reach a similar blood levels in humans as in mice. This is in line

Published OnlineFirst August 24, 2015; DOI: 10.1158/1535-7163.MCT-15-0253



with the absence of any damage in the major organs of mice treated with the drug.

In conclusion, while the ultimate proof of concept for efficacy and safety of novel oncology therapeutics lies in humans, our results, performed in relevant preclinical model of colon cancer, support further investigation of EMICORON antitumor efficacy, possibly in combination with standard chemotherapeutics or targeted compounds.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

## Authors' Contributions

**Conception and design:** M. Porru, S. Artuso, A. Bianco, M. Franceschin, A. Biroccio, C. Leonetti
**Development of methodology:** A. Orlandi, C. Leonetti
**Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.):** M. Porru, S. Artuso, M.G. Diodoro, D. Passeri, A. Orlandi, F. Savorani
**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** M. Porru, S. Artuso, E. Salvati, A. Bianco, F. Savorani, A. Biroccio, C. Leonetti
**Writing, review, and/or revision of the manuscript:** M. Porru, S. Artuso, M. Franceschin, F. Savorani, M. D'Incalci, A. Biroccio, C. Leonetti
**Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases):** E. Salvati
**Study supervision:** A. Biroccio, C. Leonetti
**Other (design and synthesis of EMICORON):** A. Bianco

## Acknowledgments

The Department of Food Science of the University of Copenhagen (Copenhagen, Denmark) is acknowledged for providing the NMR instrumentation for the metabolomics pilot study.

## Grant Support

This work was supported by grants from Italian Association for Cancer Research #14337 to C. Leonetti and #11567 and #9979 to A. Biroccio. E. Salvati has been awarded with a Post-doc fellowship from Fondazione Veronesi.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received March 30, 2015; revised August 7, 2015; accepted August 10, 2015; published OnlineFirst August 24, 2015.

## References

1. Balasubramanian S, Hurley LH, Neidle S. Targeting G-quadruplexes in gene promoters: a novel anticancer strategy? Nat Rev Drug Discov 2011;10:261–75.
2. Bryan TM, Baumann P. G-quadruplexes: from guanine gels to chemotherapeutics. Mol Biotechnol 2011;49:198–208.
3. Bochman ML, Paeschke K, Zakian VA. DNA secondary structures: stability and function of G-quadruplex structures. Nat Rev Genet 2012;13:770–80.
4. Bidzinska J, Cimino-Reale G, Zaffaroni N, Folini M. G-quadruplex structures in the human genome as novel therapeutic targets. Molecules 2013;18:12368–95.
5. Shalaby T, Fiaschetti G, Nagasawa K, Shin-ya K, Baumgartner M, Grotzer M. G-quadruplexes as potential therapeutic targets for embryonal tumors. Molecules 2013;18:12500–37.
6. Biffi G, Tannahill D, McCafferty J, Balasubramanian S. Quantitative visualization of DNAG-quadruplex structures in human cells. Nat Chem 2013;5:182–6.
7. Lam EY, Beraldi D, Tannahill D, Balasubramanian S. G-quadruplex structures are stableand detectable in human genomic DNA. Nat Commun 2013;4:1796.
8. Neidle S. Human telomeric G-quadruplex: The current status of telomeric G-quadruplexes as therapeutic targets in human cancer. FEBS J 2010;277:1118–25.
9. Li Q, Xiang JF, Zhang H, Tang YL. Searching drug-like anti-cancer compound (s) based on G-quadruplex ligands. Curr Pharm Des 2012;18:1973–83.
10. Burger AM, Dai F, Schultes CM, Reszka AP, Moore MJ, Double JA, et al. The G-quadruplex interactive molecule BRACO-19 inhibits tumor growth, consistent with telomere targeting and interference with telomerase function. Cancer Res 2005;65:1489–96.
11. Tauchi T, Shin-ya K, Sashida G, Sumi M, Okabe S, Ohyashiki JH, et al. Telomerase inhibition with a novel G-quadruplex-interactive agent, telomestatin: *in vitro* and *in vivo* studies in acute leukemia. Oncogene 2006;25:5719–25.
12. Salvati E, Leonetti C, Rizzo A, Scarsella M, Mottolese M, Galati R, et al. Telomere damage induced by the G-quadruplex ligand RHPS4 has an antitumor effect. J Clin Invest 2007;117:3236–47.
13. Leonetti C, Scarsella M, Riggio G, Rizzo A, Salvati E, D'Incalci M, et al. G-quadruplex ligand RHPS4 potentiates the antitumor activity of camptothecins in preclinical models of solid tumors. Clin Cancer Res 2008;14:7284–91.
14. Biroccio A, Porru M, Rizzo A, Salvati E, D'Angelo C, Orlandi A, et al. DNA damage persistence as determinant of tumor sensitivity to the combination of Topo I inhibitors and telomere-targeting agents. Clin Cancer Res 2011;17:2227–36.
15. Salvati E, Scarsella M, Porru M, Rizzo A, Iachettini S, Tentori L, et al. PARP1 is activated at telomeres upon G4 stabilization: possible target for telomere-based therapy. Oncogene 2010;29:6280–93.
16. Iachettini S, Stevens MFG, Frigerio M, Hummersone MG, Hutchinson I, Garner TP, et al. On and off-target effects of telomere uncapping G-quadruplex selective ligands based on pentacyclicacridinium salts. J Exp Clin Cancer Res 2013;32:68.
17. Rizzo A, Iachettini S, Zizza P, Cingolani C, Porru M, Artuso S, et al. Identification of novel RHPS4-derivative ligands with improved toxicological profiles and telomere-targeting activities. J Exp Clin Cancer Res 2014;33:81.
18. Micco M, Collie GW, Dale AG, Ohnmacht SA, Pazitna I, Gunaratnam M, et al. Structure-based design and evaluation of naphthalene diimide G-quadruplex ligands as telomere targeting agents in pancreatic cancer cells. J Med Chem 2013;56:2959–74.
19. Ohnmacht SA, Marchetti C, Gunaratnam M, Besser RJ, Haider SM, Di Vita G, et al. G-quadruplex-binding compound showing anti-tumour activity in an *in vivo* model for pancreatic cancer. Sci Rep 2015;5:11385.
20. Cuenca F, Greciano O, Gunaratnam M, Haider S, Munnur D, Nanjunda R, et al. Tri- and tetra-substituted naphthalene diimides as potent G-quadruplex ligands. Bioorg Med Chem Lett 2008;18:1668–73.
21. Franceschin M, Rizzo A, Casagrande V, Salvati E, Alvino A, Altieri A, et al. Aromatic core extension in the series of N-cyclic bay-substituted perylene G-quadruplex ligands: increased telomere damage, antitumor activity, and strong selectivity for neoplastic over healthy cells. Chem Med Chem 2012;7:2144–54.
22. Salvati E, Zizza P, Rizzo A, Iachettini S, Cingolani C, D'Angelo C, et al. Evidence for G-quadruplex in the promoter of vegfr-2 and its targeting to inhibit tumor angiogenesis. Nucleic Acids Res 2014;42:2945–57.
23. Hutchinson L, Kirk R. High drug attrition rates-where are we going wrong? Nat Rev Clin Oncol 2011;8:189–90.
24. Begley CG, Ellis LM. Drug development: raise standards for preclinical cancer research. Nature 2012;483:531–3.
25. Kurtzberg LS, Roth S, Krumbholz R, Crawford J, Bormann C, Dunham S, et al. Genz-644282, a novel non-camptothecin topoisomerase I inhibitor for cancer treatment. Clin Cancer Res 2011;17:2777–87.
26. Kurtzberg LS, Battle T, Rouleau C, Bagley RG, Agata N, Yao M, et al. Bone marrow and tumor cell colony-forming units and human tumor xenograft efficacy of noncamptothecin and camptothecin topoisomerase I inhibitors. Mol Cancer Ther 2008;7:3212–22.
27. Pessina A, Malerba I, Gribaldo L. Hematotoxicity testing by cell clonogenic assay in drug development and preclinical trials. Curr Pharm Des 2005;11:1055–65.

28. Talmadge JE, Singh RK, Fidler IJ, Raz A. Murine models to evaluate novel and conventional therapeutic strategies for cancer. Am J Pathol 2007;170: 793–804.
29. Kishimoto H, Momiyama M, Aki R, Kimura H, Suetsugu A, Bouvet M, et al. Development of a clinically-precise mouse model of rectal cancer. PLoS One 2013;8:e79453.
30. Singh M, Murriel CL, Johnson L. Genetically engineered mouse models: closing the gap between preclinical data and trial outcomes. Cancer Res 2012;72:2695–700.
31. Siolas D, Hannon GJ. Patient-derived tumor xenografts: transforming clinical samples into mouse models. Cancer Res 2013;73: 5315–9.
32. Fu XY, Besterman JM, Monosov A, Hoffman RM. Models of human metastatic colon cancer in nude mice orthotopically constructed by using histologically intact patient specimens. Proc Natl Acad Sci U S A 1991;88: 9345–9.
33. Galimi F, Torti D, Sassi F, Isella C, Corà D, Gastaldi S, et al. Genetic and expression analysis of MET, MACC1, and HGF in metastatic colorectal cancer: response to met inhibition in patient xenografts and pathologic correlations. Clin Cancer Res 2011;17:3146–56.
34. Beckonert O, Keun HC, Ebbels TM, Bundy J, Holmes E, Lindon JC, et al. Metabolic profiling, metabolomic and metabonomic procedures for NMR spectroscopy of urine, plasma, serum and tissue extracts. Nat Protoc 2007;2:2692–703.
35. Savorani F, Tomasi G, Engelsen SB. icoshift: a versatile tool for the rapid alignment of 1D NMR spectra. J Magn Reson 2010;202:190–202.
36. Ulivi P, Arienti C, Zoli W, Scarsella M, Carloni S, Fabbri F, et al. *In vitro* and *in vivo* antitumor efficacy of Docetaxel and Sorafenib combination in human pancreatic cancer cells. Curr Cancer Drug Targets 2010;10: 600–10.
37. Martin ES, Belmont PJ, Sinnamon MJ, Richard LG, Yuan J, Coffee EM, et al. Development of a colon cancer GEMM-derived orthotopic transplant model for drug discovery and validation. Clin Cancer Res 2013;19: 2929–40.
38. Rubin EH, Gilliland DG. Drug development and clinical trials-the path to an approved cancer drug. Nat Rev Clin Oncol 2012;9:215–22.
39. Ruggeri BA, Camp F, Miknyoczki S. Animal models of disease: pre-clinic animal models of cancer and their applications and utility in drug discovery. Biochem Pharmacol 2014;87:150–61.
40. Wartha K, Herting F, Hasmann M. Fit-for purpose use of mouse models to improve predictivity of cancer therapeutics evaluation. Pharmacol Ther 2014;142:351–61.
41. Teicher BA. Tumor models for efficacy determination. Mol Cancer Ther 2006;5:2435–43.
42. Baker AM, Cox TR, Bird D, Lang G, Murray GI, Sun XF, et al. The role of lysyl oxidase in SRC-dependent proliferation and metastasis of colorectal cancer. J Natl Cancer Inst 2011;103:407–24.
43. Sfaxi F, Scamuffa N, Lalou C, Ma J, Metrakos P, Siegfried G, et al. Repression of liver colorectal metastasis by the serpin Spn4A a naturally occurring inhibitor of the constitutive secretory protein convertases. Oncotarget 2014;5:4195–210.
44. Tentler JJ, Tan AC, Weekes CD, Jimeno A, Leong S, Pitts TM, et al. Patient-derived tumour xenografts as models for oncology drug development. Nat Rev Clin Oncol 2012;9:338–50.
45. Hidalgo M, Amant F, Biankin AV, Budinská E, Byrne AT, Caldas C, et al. Patient-derived xenograft models: an emerging platform for translational cancer research. Cancer Discov 2014;4:998–1013.
46. Pessina A, Albella B, Bayo M, Bueren J, Brantom P, Casati S, et al. *In vitro* tests for haematotoxicity: prediction of drug-induced myelosuppression by the CFU-GM assay. Altern Lab Anim 2002;30 Suppl 2:75.
47. Masubuchi N. A predictive model of human myelotoxicity using five camptothein derivatives and the *In vitro* colony-forming unit granulocyte/macrophage assay. Clin Cancer Res 2004;10:6722–31.
48. Pessina A, Albella B, Bayo M, Bueren J, Brantom P, Casati S, et al. Application of the CFU-GM assay to predict acute drug-induced neutropenia: an international blind trial to validate a prediction model for the maximum tolerated dose (MTD) of myelo-suppressive xenobiotics. Toxicol Sci 2003;75:355–67.

Published OnlineFirst August 24, 2015; DOI: 10.1158/1535-7163.MCT-15-0253



Molecular Cancer Therapeutics

# Targeting G-Quadruplex DNA Structures by EMICORON Has a Strong Antitumor Efficacy against Advanced Models of Human Colon Cancer

Manuela Porru, Simona Artuso, Erica Salvati, et al.

*Mol Cancer Ther* 2015;14:2541-2551. Published OnlineFirst August 24, 2015.