INTEGRATING DISCOVERY & DEVELOPMENT. **NON STOP**®

SUMMER | 2012 | **ISSUE #2**

Message from the Chairman

I am pleased to share with you Ricerca's first companywide newsletter. Ricerca has seen many changes over the past year, and I'm pleased to report that for the first half of 2011 we have achieved our expectations. Below I will introduce you to the "new Ricerca", share some of the changes and highlight what I see ahead of us.

In March of 2010, Ricerca Biosciences announced the finalization of the acquisition of [redacted] business of MDS Pharma Serv[redacted] Washington; Lyon, France; and [redacted] expanded organization makes [redacted] and provides the biopharmace[redacted] contract research organization [redacted] development process spanning discovery pharmacology (IP) through submission of an investigational new drug (IND) application and into clinical product supply. Our increased capabilities result in numerous benefits: client project drug safety assessment coordination within North America and Europe; access to cGMP and non-GMP API synthesis, chemical process development, environmental and registration services; and molecular, cellular and *in vivo* pharmacological profiling services.

In late December of 2010, I shared with Ricerca associates that as we looked into 2011, the world seemed to be somewhat brighter, and that I was confident in the "new Ricerca" despite the fact that our industry, as well as global economic and social conditions, continued to be a challenge. My confidence was based on a greater diversity of global clients, supportive investors and experienced and talented associates (and their supportive families) on three continents, actively contributing to our success.

Coming

The first half of 2011 is complete and my confidence was warranted as Ricerca's accomplishments met our expectations. Ricerca received the highest level of client awards and recognition in our history, we were recognized for excellence in client problem solving and we enhanced our industry reputation. We successfully divested a facility in Bothell, Washington enabling Ricerca to significantly improve our productivity and ISO 9001 site certification audits confirmed [redacted]. We launched and enhanced **[redacted]erca.com)** our client services [redacted]ess on harmonizing processes [redacted] introduced new capabilities to [redacted] timely basis.

I encourage you to read about the great work that continues at Ricerca and to learn about our novel approaches such as our pharmacology ecommerce system, our revolutionary OncoPanel™ high-throughput cell line for identification of anticancer drug sensitivity and resistance biomarkers, and the Dried Blood Spot (DBS) methodology now available at our Lyon facility. We've also provided an update on recent news and our planned events for the second half of 2011.

I hope you'll enjoy this newsletter.

Best regards,

Ian Lennox, *Chairman and CEO*

Study of a promising antitumor compound demonstrates Ricerca's OncoPanel platform

By Usha Warrior, Technical Director In Vitro Pharmacology, Ricerca Biosciences

Although cancer continues to be one of mankind's most devastating scourges, today there is cause for optimism. According to the American Cancer Society's Facts and Figures 2012, cancer rates have declined by 1.8 percent among men and 1.6 percent among women and the four biggest killers — lung, prostate, breast and colorectal cancer — account for the biggest declines.

Ricerca has played a long and significant role in cancer research at the discovery phase and one of the recent contributions we've made to the field is OncoPanel™, a method of screening large collections of human cancer cell lines with broad genetic heterogeneity to determine cell sensitivity and resistance to test articles.

Ricerca discovered that observing large collections of human cancer cell lines can predict biomarker associations of oncology drug response in very specific genetic subtypes. OncoPanel is a high-throughput, fully automated, screening platform to test these cell lines, enabling our clients to identify biomarkers that can be used clinically to guide treatment, as well as potentially for companion diagnostics.

Using the OncoPanel platform, Ricerca recently completed the pharmacological characterization of BI 847325, a potent, orally bioavailable compound proven to inhibit both MEK and Aurora kinases, a family of kinases involved in the regulation of mitosis. BI 847325 is currently undergoing Phase I clinical evaluation.

"Ricerca has played a long and significant role in cancer research at the discovery phase and one of the recent contributions we've made to the field is OncoPanel™."

Impact on RAS-dependent MAP kinase signaling pathway

It's well understood that the RAS-dependent MAP kinase signaling pathway plays an important role in the regulation of cell proliferation and survival. Hyperactivation of this pathway is frequently observed in human malignancies as a result of aberrant activation of receptor tyrosine kinases or gain-of-function mutations in the RAS or RAF genes.

Activating mutations in the RAS genes are found in approximately 30 percent of cancers, most frequently

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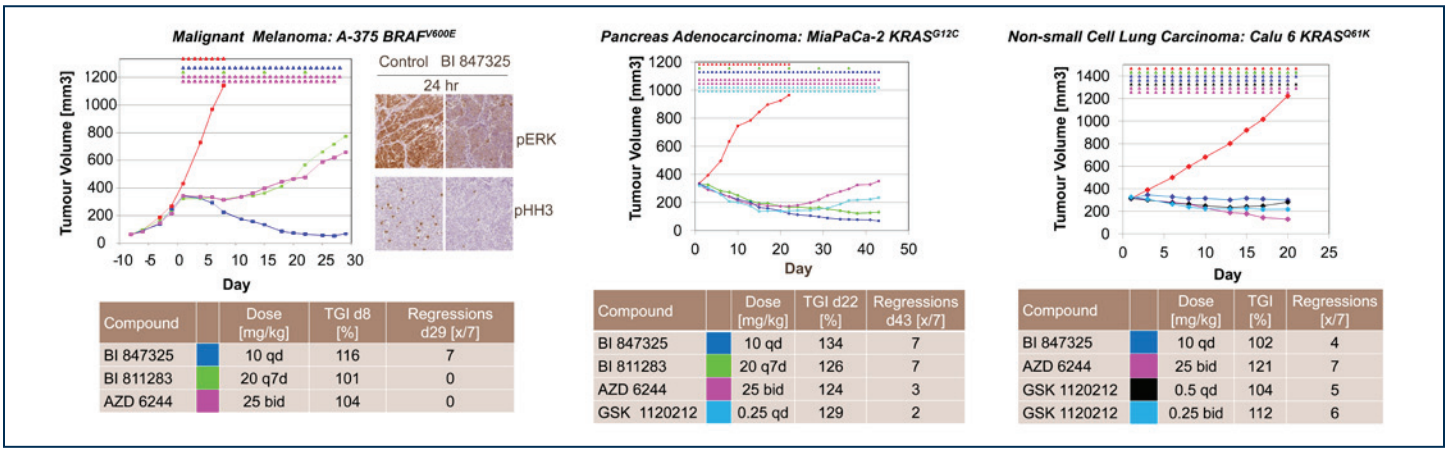
Antitumor and Pharmacodynamic Effect in Human Tumor Xenograft Models



Compound	Dose [mg/kg]	TGI d8 [%]	Regressions d29 [x/7]
BI 847325	10 qd	116	7
BI 811283	20 q7d	101	0
AZD 6244	25 bid	104	0

Compound	Dose [mg/kg]	TGI d22 [%]	Regressions d43 [x/7]
BI 847325	10 qd	134	7
BI 811283	20 q7d	126	7
AZD 6244	25 bid	124	3
GSK 1120212	0.25 qd	129	2

Compound	Dose [mg/kg]	TGI [%]	Regressions [x/7]
BI 847325	10 qd	102	4
AZD 6244	25 bid	121	7
GSK 1120212	0.5 qd	104	5
GSK 1120212	0.25 bid	112	6

Fig 1: *The in vivo efficacy of BI 847325 was assessed in standard human tumor xenograft models in nude mice and directly compared with that of the MEK inhibitors GSK 1120212 and AZD 6244 and the selective Aurora kinase inhibitor BI 811283. Tumor cells were injected subcutaneously on the flank of the animals; treatment was typically initiated when the tumors had reached a median volume of about 50 mm3 (standard setting) or about 300 mm3 (large tumor setting) and continued for 3 to 6 weeks according to the growth rate of the control tumors. Pharmacodynamic inhibition of MEK and Aurora kinase was monitored ex vivo by determining the phosphorylation state of ERK and HH3.*

Study of a promising antitumor compound *(continued)*

in KRAS. However, it has not been possible, to date, to design direct inhibitors of RAS proteins. Inhibitors of the downstream kinase MEK are active against a subset of KRAS-mutant cancers in preclinical studies, but have shown only limited success in clinical trials.

How the study was conducted

Ricerca's approach to research is a choreographed series of discrete testing steps, including biomarker modulation, cell activity and cell potency as well as studies of the effect in human tumor xenograft models.

The biomarker modulation study proved first that BI 847325 inhibits phosphorylation of both the MEK target ERK and the Aurora B target histone H3 with similar potency. When melanoma cells were exposed to increasing concentrations of BI 847325 and lysates were analyzed by Western Blot, it also proved that BI 847325 inhibits MEK and ERK phosphorylation in A375 melanoma cells in a dose-dependent manner.

To determine cell activity, cells were plated in the 384-well OncoPanel format and exposed to a range of BI 847325 concentrations. Results of these tests showed the sponsor that BI 847325 inhibits cell proliferation across indications, and that there was a trend for higher-than-average sensitivity in CRC, melanoma and pancreas carcinoma cell lines.

Potency and selectivity of BI 847325 was determined in enzymatic assays using recombinant kinases. Inhibitor concentrations were transformed to logarithmic values and the raw data were normalized. Results of these tests indicated BI 847325 is a potent inhibitor of all MEK and Aurora kinase isoenzymes.

Lastly, to determine the anti-tumor effect of the compound, the final phase of testing used standard human tumor xenograft models in nude mice and directly compared BI 847325 with MEK inhibitors GSK 1120212 and AZD 6244, as well as the selective Aurora kinase inhibitor BI 811283.

Results indicated BI 847325 showed superior efficacy compared to AZD 6244 and BI 811283 in the A375 melanoma model, and induced modulation of pERK and pHH3 in tumor samples. Against pancreas adenocarcinoma, BI 847325 induced sustained tumor regression and induced tumor regression against NSCLC.

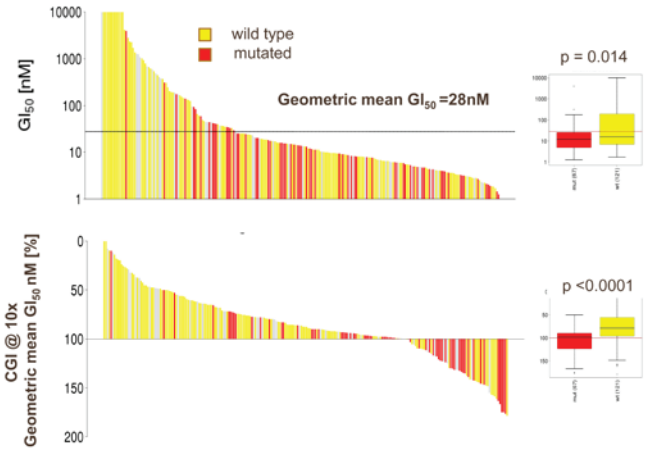
A brighter future for cancer research

In the end, BI 847325 was shown to be a potent inhibitor of cell proliferation, capable of inducing cell death in a subset of multiple cell lines. Potency indicates the compound could play a role in mutation of RAS/BRAF genes, and *in vivo* testing proved the compound inhibits growth of tumor xenografts in nude mice and induces sustained tumor regression in subsets of animals.

OncoPanel represents a new and more efficient method of discovering the most promising new compounds in the war on cancer. Ricerca is proud to make it available to the industry.

"Using the OncoPanel platform, Ricerca recently completed the pharmacological characterization of BI 847325, a potent, orally bioavailable compound proven to inhibit both MEK and Aurora kinases, a family of kinases involved in the regulation of mitosis."

Cellular Function



- RAS / BRAF mutated cell lines are significantly more sensitive than wild-type cell lines
- TP53 wt cell lines significantly cluster in the most sensitive group (geometric mean GI50 wt = 6.2 nM,mutated = 17 nM, p = 0.01;
- Among all the other genes and genes combination, additional correlations were not observed

Fig 2: *Cell lines were divided into two groups according to the mutation status of 62 single genes or 9 groups of pathway related genes. Mutation data was obtained from COSMIC (www.sanger.ac.uk/genetics/CGP/cosmic/) and in-house sequencing. For each mutation a two sample t-test was calculated, taking the log(GI50) or CGI (%) as response variables.*

Balancing speed, quality and cost of API supplies for preclinical and clinical studies

By Rich Donaldson, Vice President of Chemical Development, Ricerca Biosciences

The timing, quality of the API and cost of manufacturing the API are three competing factors with a significant impact on many drug development programs.

Recently, the main focus for many has been on cost containment, with API quality a lower priority due to the difficult financial environment in the pharmaceutical and biotech industries. Target purity for GLP toxicology is generally 97 percent; at Phase I, it should be 98 percent. Purity is generally expected to increase as the drug program advances, but often the desire is to get past proof-of-concept in human trials with the intention of out-licensing the program at Phase I.

Tight budgets may result in the bare minimum of analytical method development and often no process research. However, this is work that will need to be upgraded by Phase II, and budgeting for it at the beginning enhances the value of the overall program.

Salt and polymorph screens

A salt screen or polymorph screen should be completed prior to animal studies, but these screens are also often delayed due to budget constraints. However, this is a risky path that may cause serious delays and cost overruns later in a program if it becomes apparent that the wrong salt or polymorph had been selected. Because it is much more costly to run these screens after Phase I, we recommend performing them up front as a means of enhancing the value of a drug program. In fact, in some cases, a new salt form or polymorph may be patentable and used to extend patent life.

Process research

Some process research studies can actually reduce costs and overall timing of API manufacture. Important considerations include eliminating solid isolation steps; replacing chromatographic purifications with simpler methods; increasing reaction and work-up concentrations; eliminating strip-to-dryness steps, etc. In addition, the manufacturing process must be improved to control impurities and to consistently produce high quality API. Data from process research studies are invaluable for establishing GMP specifications for the API.

Combining production lots

For a long synthesis where a non-GMP regulatory starting material can be identified partway through the process, it may be advantageous to produce a large amount of the regulatory starting material to supply the amounts needed for GLP toxicology, GMP Phase I or other needs such as formulation lots. Producing a single GMP lot for preclinical and Phase I trials is generally a lower cost option, but it may delay preclinical studies that could make use of an easier-to-produce GLP lot. However, an advantage of utilizing a single GMP lot is that the impurity profile would be identical for preclinical and Phase I studies.

Where speed is the most important issue, it may be more appropriate to produce separate GLP and GMP lots either from a single lot of regulatory starting material or using a complete synthesis for both lots. This means not only that the API would be available more quickly for toxicology studies, but that there would be reduced risk to production of the GMP lot because of process experience gained from the GLP lot synthesis. From a quality standpoint, production of two separate API lots yields two full analytical datasets to help with establishing appropriate GMP specifications at Phase I.

"Target purity for GLP toxicology is generally 97 percent; at Phase I, it should be 98 percent."

Multiple options

There are multiple options available for managing speed, quality and cost of API manufacturing as part of an overall drug development program. The best strategy will be dependent upon the specific priorities of the program as well as the ease of API production.

Typically, if an API development program is designed and executed at preclinical or Phase I, the rest of the development process through to commercialization will readily fall into place, minimizing costly surprises. Most of the manufacturing strategy for a full drug development program should already be defined by the time the IND is approved. Having a complete package of API studies at Phase I is an important advantage in moving the program to Phase II and beyond.

Drug Safety and Metabolism ICH guidelines eased for women of child bearing potential

By Jeanne Stadler, Consultant Toxicology and Edward Marsden, Associate Director of Toxicology, Ricerca Biosciences

Enrolling women of child bearing potential (WOCBP) has long posed an ethical dilemma for the conduct of clinical trials. It was reasoned that if a study compound has not successfully undergone developmental and reproductive toxicology (DART) testing, there is risk of a fetus being exposed to a compound with potentially damaging consequences.

In 1997, the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) published a guideline known as M3(R1) that addressed principles for the development of non-clinical strategies on the timing of toxicity studies in relation to the conduct of clinical trials.

Until 2009, the ICH recommended that studies for the enrollment of women of child bearing potential in the EU and Japan include the conduct of an embryo-fetal development study; in addition, a (female) fertility study was required for Japan. Enrollment of WOCBP was easier in the United States since patients might be recruited without these studies, providing that "appropriate precautions… (are taken) to minimize the risk."

Fig 1: *Hiciliquis mo duntia num veni con estibusdae duciisci totas sus et asi tem. Nam ea desseque dolo erumquam endem voluptatur rerum unt est ab int assunto temqui od experum labori alit incturi onsero dist, consedi utet faccumq uibusam*

Ricerca also recommends adding a toxicokinetics (TK) profile determination in these improved preliminary studies in order to compare expected exposure in animals and humans to better estimate safety factors and more accurately define a starting dose in the population of WOCBP.

The second revision

The second revision of the guideline (M3(R2)) in 2009 broadened, simplified and clarified the cases where WOCBP could be recruited in clinical trials with or sometimes without limited DART studies, depending on the stage of development of the test compound. Although the ICH guideline still acknowledges the need to protect WOCBP from unexpected exposure of her embryo or fetus, the non-clinical principles and prerequisites are now harmonized between the three ICH regions.

The enrollment of WOCBP is now possible without conducting embryo-fetal development studies if the following conditions are met:

- If clinical trials are of short duration, i.e., limited to two weeks, and intensive control of pregnancy risk such as pregnancy testing prior to the start of the study and contraception (single or double) is maintained.
- WOCBP can also be included if the disease under study is predominant in women and the objectives of the clinical trial cannot be effectively met without inclusion of WOCBP. Again, intensive control of pregnancy risk must be maintained throughout the study.

Although the guideline contains additional examples under which DART studies may not be required to support early clinical trials with WOCBP, the long list of required conditions and ethical concerns would mean that only a very limited number of molecules would qualify for such exemptions.

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Drug Safety and Metabolism ICH guidelines eased for women of child bearing potential *(continued)*

"It is hoped that the revised guideline will expedite the development of new pharmaceuticals for safe use during pregnancy and also allow a more intelligent use of animals prior to the clinical proof of concept."

Preliminary development studies

More promising for the inclusion of WOCBP in clinical trials is the addition of preliminary development studies. If the results of improved and well-conducted preliminary embryo-fetal development studies in two species do not show any adverse effect on the developing embryo or fetus following administration during organogenesis, enrollment of a maximum of 150 WOCBP for up to three months is possible. This assumes that the usual provisions regarding contraception and verification of the absence of pregnancy have been fulfilled prior to the start of the clinical trial.

The guideline gives broad indications for the design of these improved preliminary studies: six females per group; treatment during organogenesis; C-section close to the end of pregnancy; recording of reproductive parameters and external and visceral examination of fetuses.

Ricerca also recommends adding a toxicokinetics (TK) profile determination in these improved preliminary studies in order to compare expected exposure in animals and humans to better estimate safety factors and more accurately define a starting dose in the population of WOCBP. What we have just described can only be applied for early clinical trials and only with a limited number of women.

DART studies in later-phase trials differ

The DART package requirements for later clinical trials and marketing authorization remain slightly different for the three ICH regions.

In the United States, assessment of embryo-fetal development can be deferred until Phase III for WOCBP, assuming precautions are in place to prevent pregnancy in clinical trials. In the EU and Japan, other than the situations described above, definitive, so-called "classical" non-clinical embryo-fetal developmental studies must be completed before exposure of WOCBP.

Fig 2: *Hiciliquis mo duntia num veni con estibusdae duciisci totas sus et asi tem. Nam ea desseque dolo erumquam endem voluptatur rerum unt est ab.*

In all ICH regions, WOCBP can be included in repeat-dose Phase I and II trials before conduct of the female fertility study because an evaluation of the female reproductive organs is performed in repeat-dose toxicity studies of at least of two weeks duration. Studies of female fertility should complete non-clinical (segment 1) tests in order to support inclusion of WOCBP in large-scale or long-duration clinical trials. In all ICH regions, a pre-postnatal development (PPND) study must be submitted for marketing approval. Here again, however, there are cases where conducting a PPND study should be advanced, for example when there is a need for a pediatric indication.

Ultimately, all female reproduction toxicity studies and the standard battery of genotoxicity tests should be completed before inclusion of WOCBP in any clinical trial not using highly effective birth control or where the pregnancy status is unknown.

Conclusion

The revised ICH M3(R2) guideline opens opportunities for an earlier inclusion of women of child bearing potential in clinical trials providing that the safety of these women remains paramount. These clinical trials must be of a short duration (maximum of three months) and involve a limited number of women (no more than 150). These studies can be performed either in absence of any non-clinical DART study (but according to very restricted conditions) or, more likely, supported by a reduced but well-designed package of preliminary DART studies in two species.

It is hoped that the revised guideline will expedite the development of new pharmaceuticals for safe use during pregnancy and also allow a more intelligent use of animals prior to the clinical proof of concept.

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Upcoming Events 2011

Japanese Society of Toxicology
Sendai, Japan
July 17-19, 2012

European Teratology Society
Linz, Austria
September 2-5, 2012

Informa AgChem
Barcelona, Spain
September 5-6, 2012
Booth #18

ChemOutsourcing
Long Branch, NJ
September 10-13, 2012

11th Annual Biotech in Europe Investor Forum
Hilton Zurich Airport Hotel
Zurich, Switzerland
October 1-2, 2012

Safety Pharmacology Society
Phoenix, AZ
October 2-4, 2012
Booth #128

News

Dr. G. Lynn Miesel Named New Ricerca Technical Director

Dr. G. Lynn Miesel, a respected veteran in the research industry, has been named the new technical director for Ricerca Biosciences. In her new role, she will utilize more than 13 years of industry experience in anti-infectives to lead projects for Ricerca clients. Dr. Miesel's experience in the pharmaceutical industry includes expertise in genetics, biochemistry, microbiology and infectious diseases drug discovery. She has extensive experience in project design and leadership, including international external collaborations. Prior to joining Ricerca, Dr. Miesel worked for Merck Research Laboratories as the biology collaboration lead of infectious disease research. While there, Dr. Miesel focused on project conception, design and coordination for target validation, lead identification and lead optimization programs. She successfully advanced two antibacterial lead identification programs and designed a lead identification program with BioRelix that led to a licensing deal. Dr. Miesel serves as an ad hoc grant reviewer for the National Institutes of Health and has coauthored 16 peer-reviewed publications.

Ricerca Publishes Case History on IND Enabling Studies and Chemistry for Virtual Company

Ricerca recently completed a project for a virtual pharmaceutical company that required chemistry and drug safety services to file an IND application for an in-licensed anticancer compound. A case history of the project has been developed and made available to interested parties. The case history details the situation and its solutions. Although some early proof-of-concept work had been done, including extensive human tumor cellular testing, both the timeline and budget were extremely tight to demonstrate oral bioavailability; upgrade synthesis process to production scale; develop the CMC section for IND filing; and conduct concurrent safety, toxicology and DMPK studies for IND filing. The case history describes the approach Ricerca took to each challenge as well as the successful outcome.

Ricerca Scientists Win in GE IN Cell Analyzer Image Competition

High Content Analysis (HCA) is the automated process of extracting and analyzing quantitative data from cell images that have been captured with a high-resolution light microscope (usually a fluorescence microscope) equipped with a sensitive camera. This powerful new technology is having an impact across many disciplines including biotechnology, drug discovery and drug development and safety testing. Because these images are often inherently beautiful, GE sponsors an annual competition to find the best for a calendar and screen saver collection. This year, Ricerca is proud to announce that two of our own scientists, Karen Bernards and Phuong Nguyen, were among the winners in the popular competition.