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RNA Interference and Personalized Cancer Therapy

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Abstract: Despite billions of dollars allocated to cancer research, cancer remains the number 2 cause of death in the United States with less than 50% of advanced cancer patients living one year following standard treatment. Cancer is a complex disease both intrinsically and in relation to its host environment. From a molecular standpoint no two cancers are the same despite histotypic similarity. As evidenced by the recent advances in molecular biology, treatment for advanced cancer is headed towards specific targeting of vulnerable signaling nodes within the reconfigured pathways created by "omic" rewiring. With advancements in proteo-genomics and the capacity of bioinformatics, complex tumor biology can now be more effectively and rapidly analyzed to discover the vulnerable high information transfer nodes within individual tumors. RNA interference (RNAi) technology, with its capability to knock down the expression of targeted genes (the vulnerable nodes), is moving into the clinic to target these nodes, which are integral to tumor maintenance, with a low risk of side-effects and to block intrinsic immunosuppressors thereby priming the tumor for immune attack. An RNAi based sequential approach, a so called "one-two punch," is being advocated comprising tumor volume reduction (ideally to minimal residual disease status) effected by integrated multi-target knockdown followed by immune activation. Examples and recent developments are provided to illustrate this highly powerful approach heralding the future of personalized cancer therapy. [*Discovery Medicine* 15(81):101-110, February 2013]

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Introduction

Despite the progress made in diagnostic capabilities and therapeutic armamentarium over the forty years since the National Cancer Act of 1971 was signed into law on December 23, 1971, cancer remains the number 2 cause of death in the United States (just below heart disease). Half of men, a third of women, and 1 in 330 children will fight cancer in their lifetime and half of Americans will have cancer when they die. Benefits from the three major treatment approaches have now reached a glass ceiling. In 1971, less than 50% of advanced cancer patients lived 1 year and today after hundreds of billions spent still less than 50% of advanced cancer patients live 1 year while undergoing standard treatment. Moreover, surviving five years is a rare event. Importantly, based on major changes in our understanding of the cancer process and information theory, it is now recognized that, like their hosts, no two cancers are the same; the genetic/epigenetic alterations in different cancers are diverse and heterogeneous resulting in unique ecosystems including tumor cells in symbiotic relationship with the recruited surrounding cells (Egeblad *et al.*, 2010; Floor *et al.*, 2012). This explains why two patients with the same cancer, disease stage, and treatment often have very different responses ranging from complete response to rapidly progressive disease. The reason for the variance in response is the inability to target the rewired core mechanisms associated with each individual's cancer. The ability to do so is truly "personalized cancer therapy": the new frontier of cancer medicine.

Hanahan and Weinberg (2000) initially proposed six hallmarks of cancer comprised of those tumor-supporting biologic capabilities resulting from genetic/epige-

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netic changes; they are: self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, evasion of programmed cell death, limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis. They have recently added two additional hallmarks: the reprogramming of energy metabolism and evasion of immune destruction (Hanahan and Weinberg, 2011). Therefore, in the face of this evolved, functionally rewired biologic infrastructure, including signaling pathway cross-talk and redundancy, it is not surprising that the next generation of cancer therapy must consist of an individually tailored, multipronged approach targeting multiple specified central gene nodes in order to dismantle the complexity that is the cancer process.

Recent advances in proteo-genomics have led to the development of network models that integrate intra- and inter-pathway cellular signaling interactions potentially allowing the differentiation between cancer and non-malignant states. Tumor genomes can now be sequenced by next generation sequencing and thereafter analyzed (Mardis, 2012; Morozova and Marra, 2008; Pleasance *et al.*, 2010). For example, through next generation sequencing, genetic analysis was performed on 24 pancreatic cancers from which 20,661 protein-coding genes were analyzed and 63 genetic alterations in 12 cellular signaling pathways and processes were found (Jones *et al.*, 2008). In addition, genome sequence analysis comparing primary to metastatic pancreatic cancer sites reveals that genome instability with rearrangements and amplifications as well as mutations persist after cancer dissemination, with parallel, convergent, and divergent organ-site specific evolution (Campbell *et al.*, 2010). The mathematical model analysis of pancreatic cancer tumor DNA sequence data suggests it takes more than 10 years of evolution from an initiating mutation to the birth of a non-metastatic "cancer" parental clone and an additional 5-6 years to develop metastatic potential (Yachida *et al.*, 2010). An exome sequencing analysis with a larger cohort of early (stage I and II) sporadic pancreatic ductal adenocarcinoma (PDAC) reaffirmed 16 known significantly mutated genes and uncovered novel mutated genes involved in chromatin modification, DNA damage repair, and mechanism of the embryonic regulators of axon guidance (Biankin *et al.*, 2012). Divergent and yet recurrently mutated genes recapitulated clustering in core signaling pathways in pancreatic cancer. In the not too distant future, complete tumor DNA sequence (and copy number alterations) will be obtained in a timely fashion at a reasonable cost followed by bioinformatics analysis resulting in an essential bio-function fingerprint of a patient's cancer that will enable construction of an individualized targeted therapy.

It is our premise that identification and directed, integrated, bioinformatics-based "personalized" targeted therapy will be the foundation of future management of cancer. Although not all potential therapeutic targets are druggable by the traditional pharmaceutical approach (Drews, 2000; Verdine and Walensky, 2007), with the discovery of RNA interference (RNAi) technology, potentially all identifiable genes can be targeted for gene expression knockdown (Burnett and Rossi, 2012; Rao *et al.*, 2009). RNAi is a natural process through which expression of a targeted gene or a set of targeted genes is dampened with high specificity and selectivity. We propose that through the compilation of a patient tumor genome database as part of a multiomic compilation, i.e., an integrated genome-gene expression (proteome) based network that recognizes the level of connectivity of pathways, processes, and central information integrative genes (hubs), a more-effective anti-tumor therapy based on tumor genome analysis can be identified (Albert *et al.*, 2000; Bild *et al.*, 2006; Hanahan and Weinberg, 2000; Jeong *et al.*, 2001). Identification of dominant biorelevant pathways and key hubs will expose the cancer cells' "Achilles' heel," the fragile sites within an otherwise robust system (Albert *et al.*, 2000; Carlson and Doyle, 2002; Hartwell *et al.*, 1997) which represent the cost of phenotypic adaptation (Weinstein, 2002). These analytics are the basis of a targeted RNAi knockdown strategy (Elbashir *et al.*, 2001; Ichim *et al.*, 2004; Lakka *et al.*, 2004; Press *et al.*, 1992) as the core of a personalized approach to cancer evaluation and treatment. In addition to direct tumor cell targeted therapy, the RNAi strategy can also be readily utilized to inhibit tumor cell-microenvironment crosstalk, e.g., knockdown of immune-suppressive targets with autologous vaccine approach (Senzer *et al.*, 2012b). The safety of an RNAi therapeutics will need continuing assessment in biorelevant preclinical models and in phase I clinical trials. However, fledgling siRNA-based clinical trials focused on cancer have not encountered significant adverse effects and evaluation of consequent siRNA protein effects shows very limited off-target effects (Table 1) (Aleman *et al.*, 2007; Wang *et al.*, 2011). In response to theoretical and observed limitations in clinical application of viral vectors/particles for RNAi delivery, non-viral delivery vehicles are being aggressively evaluated emphasizing safety, enhanced focused biodistribution, continued efficacy with repeated administration, documentation of transgene expression at the tumor site, and functional activity appropriate for clinical application (Pirollo *et al.*, 2008; Templeton *et al.*, 1997). In the remainder of this review, we will briefly review the current status of

the clinical development of RNAi based cancer therapeutics and comment on their future development.

RNAi and Mediators of RNAi

A naturally-occurring process of gene regulation and defense, RNAi controls the expression of intrinsic genes and the timing of morphogenesis in embryonic development (Fire *et al.*, 1998; Lee *et al.*, 1993) whereby small sequences of intrinsic antisense RNA, microRNA (miRNA, miR), or extrinsic double-stranded RNA (dsRNA) (variable amongst different organisms) trigger repression of targeted-gene expression. The technological application of introduction of synthetic small interfering RNA (siRNA) molecules into cells results in target gene messenger RNA (mRNA) degradation or p-body sequestration for translational repression. The RNAi process starts with loading of double-stranded siRNA onto ATP-dependent RNase III enzyme Dicer to trim the siRNA to 22 nucleotides segments and to form the initiation complex. With the assistance of Dicer, double-stranded siRNA is loaded onto an Argonaute (Ago) protein-containing "RNA interfering silencing complex" (RISC) where one strand of the siRNA (the passenger strand) is released with the assistance of ATP-dependant helicase leaving the embedded guide strand (Ichim *et al.*, 2004). Loaded RISCs then seek out a complimentary "target" sequence resulting in mRNA cleavage, degradation, and sequestration. Endonucleolytic cleavage of the target mRNA occurs at a single site in the center of the target mRNA-siRNA antisense strand duplex (Elbashir *et al.*, 2001) and is mediated by Ago 2 (Liu *et al.*, 2004). Thus, once determined, the genotypic and phenotypic differences between cancer and normal cells make the use of RNAi appealing since it's highly specific and has been used in numerous cancer-related studies both for validating the role of a specific target gene and for both *in vitro* and *in vivo* demonstration of its effectiveness as a therapeutic target.

The action of RNAi can be mediated either through chemically synthesized siRNA or short hairpin RNA (shRNA) transcribed from a DNA expression vector. DNA-based shRNA and RNA-based siRNA are intrinsically different types of molecules, thus their delivery into cells, pathways utilized for RNA interference, and potential off-target effects are also different. Although both types of molecules are capable of effecting target-specific gene knockdown, each type of molecule has its comparative advantages and disadvantages.

Table 1. Clinical Trials of RNAi-Based Cancer Therapeutics* (Wang *et al*., 2011).

Drug	CALAA-01	ALN-VSP02	TKM-080301	ATN-RNA	Atu027	EZN-2968	FANG™ Vaccine
Phase	I	I	I	I	I	I	I
Tumor type	solid tumor	advanced solid tumor with liver involvement	solid tumor or lymphoma	astrocytic tumor	advanced solid tumor	advanced solid tumor or lymphoma	advanced solid tumor
Delivery	cyclodextrin-containing polymer nanoparticle	lipid nanoparticle	lipid nanoparticle	naked	lipoplex	naked	*ex-vivo* by electroporation
Route	IV infusion	IV infusion	IV infusion	local, brain	IV infusion	IV infusion	intradermal
Target	ribonucleotide reductase M2 (RRM2)	kinesin spindle protein and VEGF	polo-like kinase 1 (PLK1)	tenascin-C	protein kinase N3	HIF-1 alpha	furin
RNAi	unmodified siRNA	chemically modified siRNA	chemically modified siRNA	160bp double-stranded RNA	chemically modified siRNA	LNA antisense oligonucleotide	bi-shRNA
Current enrollment	36	41	42	53	33	59	59
Company	Calando	Alnylam	Tekmira	Polish Academy of Sciences (Poland)	Silence Therapeutics AG (Germany)	Enzon	Gradalis
Reference	Templeton *et al*., 1997, a	Cervantes *et al*., 2011, a	a	Rolle *et al*., 2010	Santel *et al*., 2010, a	Patnaik *et al*., 2009, a	Senzer *et al*., 2012b, a

Note: a, www.clinicaltrials.gov; *, updated as of Sept. 7, 2011.

Recently, we developed a new class of RNAi, bi-functional shRNA (bi-shRNA), that is able to effectively take advantage of multiple natural RNAi mechanisms for more potent and durable target-gene knockdown (Rao *et al.*, 2010). The bi-shRNA strategy is schematically presented in Figure 1. Bi-shRNA was developed to exploit both the post-transcriptional mRNA cleavage and translational inhibition mechanisms of RNAi (Rao *et al.*, 2009). It consists of two stem-loop shRNA structures: one cleavage-dependent unit with perfectly matched passenger- and guide-strand, and one cleavage-independent unit composed of a strategically mismatched double strand. The two shRNA units are embedded in miR-30 scaffold and are encoded by a plasmid vector. The mature transcript of the cleavage-dependent unit is loaded onto cleavage-dependent RISC (due to Ago 2 intrinsic endonuclease activity), whereas the processed transcript of the cleavage-independent unit, by virtue of the embedded stem-loop mismatch, binds to the cleavage-independent RISC (Ago 1, 3, and 4 without endonuclease activity and Ago 2 in which the endonuclease activity is blocked by the mismatch) and inducing translational inhibition. In principle, bi-shRNA is able to induce cleavage, degradation of the target mRNA, and translational inhibition concurrently, leading to more rapid onset of gene silencing,

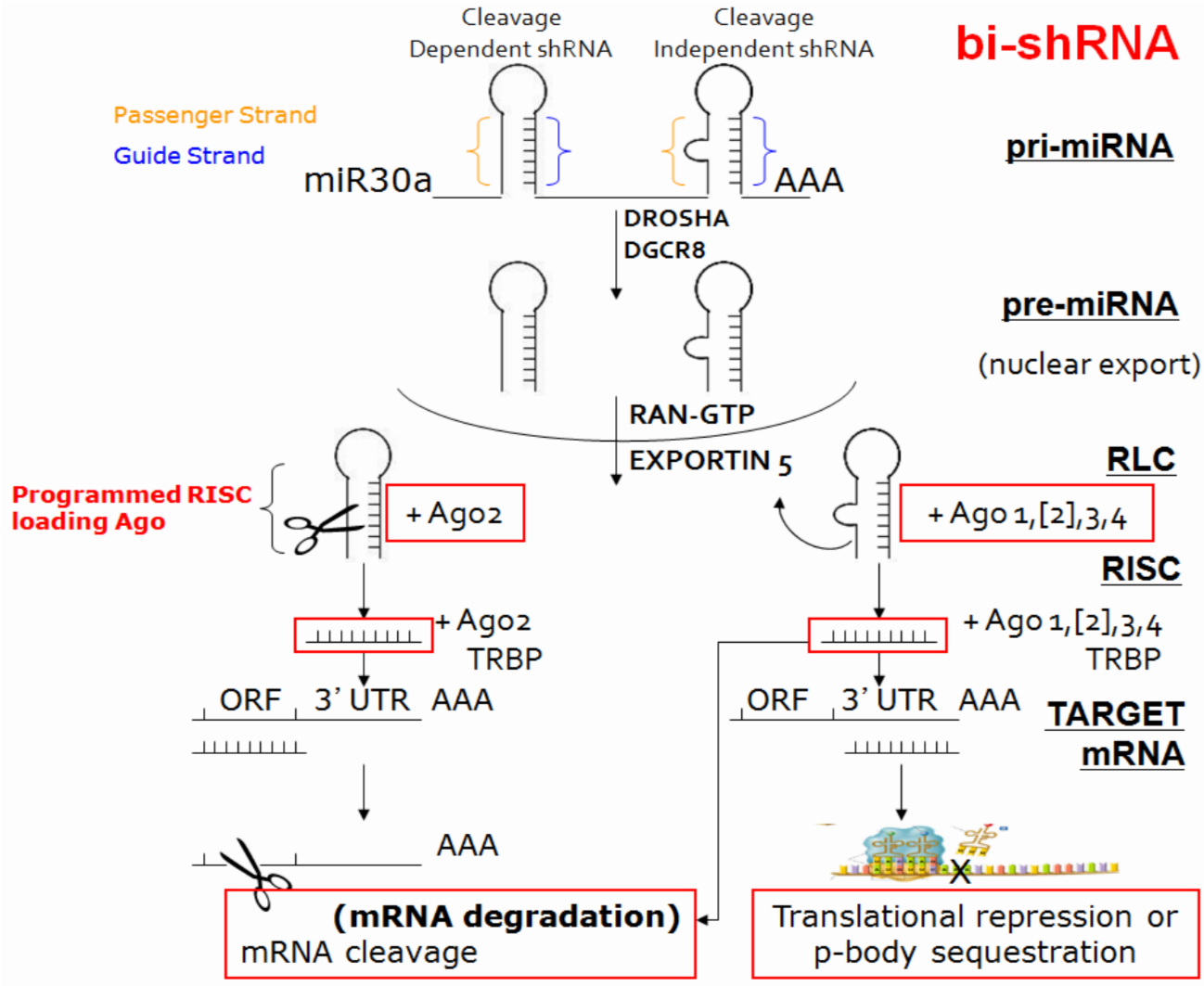

Figure 1. Schematic of the bi-functional shRNA concept. The bi-functional shRNA concept is to express two stem-loop shRNAs for each targeted mRNA; one with perfect matching stem sequences, one with mismatches at the central location (bases 9-12) and additional location of the stem. The purpose of the bi-functional design is to promote loading of mature shRNA onto both cleavage-dependent and cleavage-independent RISCs, so that the expression of target mRNA can be more effectively and efficiently shut down both through target mRNA degradation and translation repression mechanisms.

higher efficacy and greater durability when compared with siRNA and greater specificity compared to miRNA insofar as the cleavage-independent bi-shRNA guide strand is fully complementary to the mRNA target sequence.

Due to their robustness and specificity, siRNA and shRNA have been extensively used to silence tumor-related targets. A number of preclinical studies have demonstrated favorable outcomes by silencing genes critical for tumor cell growth, metastasis, angiogenesis, and chemoresistance (Phalon *et al.*, 2010). Despite the immense potential of RNAi in clinical applications, several hurdles have to be overcome for RNAi-based therapies to move from the bench to the clinic. First, efficient and differential tumor delivery and cell uptake is required. Second, due to sequence-independent effects, RNAi can induce an innate immune response, which is a particular concern when considering siRNA. In addition, off-targeting effects have to be carefully measured since RNAi has the potential to knock down non-targeted genes via sequence-dependent/RISC-mediated effects. Moreover, exogenous siRNA and shRNA could saturate endogenous miRNA pathways via sequence-independent/RISC-mediated effects and elicit cytotoxicity. Lastly, the molecular mechanism and pharmacokinetics data have to be profiled for regulatory filings. Those issues have been extensively reviewed by us and others (Rao *et al.*, 2009; Tiemann and Rossi, 2009; Wang *et al.*, 2011). Nonetheless, the application of RNAi for cancer therapy is cautiously being investigated in early phase clinical trials.

RNAi-based cancer therapeutics in clinical trials

Calando Pharmaceuticals reported their study results in human patients with solid tumors in March of 2010 (Davis *et al.*, 2010), which is believed to be the first proof-of-concept study for efficacy in target gene knockdown with systemically administered siRNA in human. In this study, CALAA-01, an unmodified siRNA targeting ribonucleotide reductase M2 (RRM2) was formulated with cyclodextrin-containing polymer nanoparticles, decorated with transferrin (Tf), and administered intravenously to patients with metastatic melanoma. siRNA-loaded nanoparticles accumulated in tumor cells in a dose-dependent manner. A statistically significant reduction of both RRM2 mRNA and protein was found when compared with pre-dosing tumor tissues. The predicted cleavage product of RRM2 mRNA was detected from one patient who received the highest dose of siRNA nanoparticles (30 mg/m^{-2}). Furthermore, the safety profiles showed that the administered siRNA was well tolerated and the dose-limiting toxicities were absent. Despite the promising data, the effects on tumor reduction or clinical phenotypes were unknown since the clinical study was still ongoing at the time of publication.

Alnylam Pharmaceuticals has developed chemically modified siRNA to treat a variety of diseases including cancer, some of which are currently in phase I or II clinical trials. Chemically modified siRNA was formulated using stable nucleic acid-lipid particle (SNALP) technology developed by Tekmira Pharmaceuticals for delivery. Two chemically modified siRNAs, each one targeting either the kinesin spindle protein (KSP) or vascular endothelial growth factor (VEGF)-A, were formulated together (ALN-VSP02) for systemic intravenous (IV) administration. ALN-VSP02 was developed to treat advanced solid tumors with liver involvement due to SNALP's high delivery efficiency to the liver. Thirty-seven patients, a majority of them with colorectal cancer, were administered ALN-VSP02 by a 15-minute infusion once every two weeks with doses ranging from 0.1 to 1.5 mg/kg until disease progression. This treatment was well tolerated in most patients following premedication with steroids, H1 and H2 blockers, and acetaminophen. Seven of 37 patients with stable disease or better after 4 months were continued on an extension study at 1.0 mg/kg or 1.25 mg/kg. Tumor types included head and neck squamous cell carcinoma, angiosarcoma, endometrial cancer, renal cell carcinoma (RCC), and pancreatic neuroendocrine tumor (PNET). Target knockdown for VEGF was demonstrated by 5' RACE (Rapid Amplification of 5′ cDNA Ends) evaluation of post treatment tumor biopsies at 0.4 mg/kg; knockdown for KSP was not able to be determined because of low expression levels. As last reported at the 2012 ASCO meeting, 3 patients remain on study, including an endometrial cancer patient with an ongoing partial response (PR) who has had >80% tumor regression after 19 months of treatment and two patients with RCC and PNET with continued stable disease (SD) after nearly 1 year of treatment; all patients initially had overexpression of VEGF. Adverse events for patients under treatment included fatigue or elevated alkaline phosphatase; a decrease in spleen volume likely due to an on-target effect of KSP knockdown was not associated with any adverse effect. A phase II study is planned at a dose of 1 mg/kg targeting endometrial cancer, RCC, PNET, and hepatocellular carcinoma patients with VEGF overexpression tumors.

Tekmira Pharmaceuticals has also developed an oncology product candidate, TKM-PLK1, utilizing the SNALP delivery technology. TKM-PLK1 targets PLK1 (polo-like kinase 1), a protein involved in tumor cell proliferation. Inhibition of PLK1 expression prevents the tumor cell from completing cell division, resulting

in cell cycle arrest and death of the cancer cell. TKM-PLK1 has been shown in preclinical animal studies to selectively kill cancer cells. A single, systemic intravenous administration of TKM-PLK1 blocked PLK1 expression in liver tumors causing extensive tumor cell death. After repeat dosing, this result translated into significant inhibition of tumor growth and prolonged survival without evidence of toxicity (http://www.tekmirapharm.com/Programs/Products.asp). In December 2010, Tekmira initiated a phase 1 human clinical trial of TKM-PLK1, an open label, multi-dose, dose escalation study designed to evaluate the safety, tolerability, and pharmacokinetics of TKM-PLK1 as well as determining the maximum tolerated dose. Secondary objectives of the trial include measurement of tumor response and pharmacodynamic assessments. Tekmira has since released interim results from the TKM-PLK1 phase 1 study showing that TKM-PLK1 was generally well tolerated. Twenty-one patients have been treated at doses ranging from 0.15 mg/kg to 0.90 mg/kg. Patients are dosed once weekly with each cycle consisting of three doses followed by an off-week. Thus far, one patient has achieved a partial response and continues treatment at 0.6 mg/kg having received 15 doses over 5 months. Another patient attained stable disease and completed six cycles of treatment at 0.6 mg/kg over 6 months. Patient enrollment is continuing at 0.75 mg/kg.

A group from Poland reported the results of local administration of naked 160 base-pair double stranded RNA targeting tenascin-C (TN-C) directly into the infiltrative neoplastic tissue during surgery of glioma (Rolle *et al.*, 2010). Overexpression of the extracellular matrix protein TN-C contributes to adhesion, invasion, migration, and proliferation of tumor cells in glioma. Forty-six patients with brain tumors, including grades II and III glioma and glioblastoma multiforme (GBM; grade IV), received 80 μg of unmodified double-stranded RNA carried by calcium chloride. The median length of overall survival was 106.6 weeks for patients treated with double-stranded RNA, compared with 48.2 weeks without treatment as observed in a previous study. Of note, the median overall survival for patients with grade III glioma and GBM were 72.3 weeks and 66.7 weeks, respectively, compared with 59.1 weeks and 52.8 weeks for patients after standard brachytherapy. No significant neurological toxicities observed.

Silence Therapeutics has recently begun a phase I study to address the safety, tolerability, and pharmacokinetics of Atu027. Atu027 contains a chemically modified 23 base-pair blunt-end siRNA targeting protein kinase N3 (*PKN3*), which was found to be a novel downstream target of PI-3 kinase in vascular and endothelial cells (Collazos *et al.*, 2011). Atu027 suppression of PKN3 expression leads to robust inhibition of both lymphogenous as well as hematogenous metastases. The delivery vehicle is a liposome complex consisting of a cationic lipid, a neutral helper lipid and a pegylated lipid (Atuplex), which is exclusively taken up by endothelial cells of essentially all vascular beds (Strumberg *et al.*, 2012). Mice with subcutaneous prostate cancer xenografts treated with Atu027 showed an average tumor volume of less than half that seen in mice injected with a sucrose solution. Treated mice also showed lymph node metastases less than half of the time mice treated with sucrose did, with a third of the volume. Interestingly, however, in the spontaneous mouse metastasis model, despite a reduction in pulmonary metastases, the primary tumor was not so affected (Santel *et al.*, 2010). Atu027 was tested for toxicity in *Cynomolgus* monkeys at doses of 0.3, 1.0, and 3.0 mg siRNA/kg every fourth day. *PKN3* gene expression levels were determined from lung tissue taken from the animals after the last dose and silencing was observed for all three doses at a significant level as compared to sucrose treated animals. 0.3 mg siRNA/kg was determined to be the lowest active dose (Aleku *et al.*, 2008). Patients with advanced or metastatic solid tumors in a phase I trial were given a single 4-hour IV infusion of designated dose with a three-week follow-up before receiving twice weekly dosing for an additional four weeks with continued treatment in case of SD. Thirty-four patients have been treated and the trial is ongoing in the last (10^{th}) dose level. No premedication was used. One dose limiting toxicity occurred at dose level 10 (increase of lipase, grade 3), thus the maximum tolerated dose is at 0.336 mg/kg. Robust reduction of soluble VEGF-R1 (sFLT-1) was observed based on the effective target knockdown dose range determined in *Cynomolgus* primate studies, establishing VEGF-R1 as a potential surrogate biomarker for target knockdown. SD response for three and six months after treatment was observed in 10 and 3 patients, respectively. Two patients with neuroendocrine cancer had disease stabilization for 9 and 12 months. Partial regression of pulmonary metastases was found in 1 patient. Another patient with breast cancer had regression of liver metastases.

Our first clinical experience with the bi-shRNA platform involved the *ex vivo* knockdown of furin, a Ca^{2+}-dependent, non-redundant proprotein convertase that is essential for proteolytic maturational processing of immunosuppressive TGF-β isoforms (β_1 and β_2). FANG™ (*F*urin-knockdown *An*d *G*M-CSF-augmented) is an autologous whole cell cancer vaccine, a prototype of the 'triad' immunotherapy incorporating 1) broad antigen presentation, 2) GM-CSF-based afferent

immunostimulation, and 3) inhibition of intrinsic immunosuppressive proteins (TGF-β_1 and β_2) (Kumar *et al.*, 2012; Senzer *et al.*, 2012a). Harvested, autologous cancer cells are transfected with the GM-CSF/bi-shRNA^{furin} (FANG) expression plasmid via electroporation (Kumar *et al.*, 2012; Senzer *et al.*, 2012a). A phase I clinical trial (BB-IND 14205) involving 52 cancer patients was recently completed (Senzer *et al.*, 2012b). Results demonstrated better than 90% knockdown of both the bi-shRNA target, furin, and the furin processed TGF-$\beta_{1,2}$, thereby confirming the mechanistic expectation of this novel RNAi platform. Moreover, predicted extensive GM-CSF expression verified our ability to successfully construct multi-cassette vectors with GMP manufacturing techniques fulfilling FDA requirements for clinical testing. Twenty-seven patients received ≥1 vaccine dose and 23 achieved SD as their best response. No toxic effect was identified. Median survival of the FANG™ treated patients from time of procurement was 554 days and has not been reached from time of treatment. Expected survival of similar patients is historically less than 1 year. Sequential ELISPOT analysis revealed a dramatic and significant increase in immune response from baseline to month 4 in half of the FANG™ treated patients. Comparison of survival between ELISPOT positive and ELISPOT negative patients demonstrated a statistically significant increase in survival from time of procurement (p=0.045) and time of treatment (p=0.025). These phase I study results demonstrate mechanism and safety and provide preliminary suggestion of effectiveness of the bi-shRNA technology and clinical functionality of a multi-component DNA expression vector. Three phase II studies, which are ongoing (BB-IND14205), were considered justified by FDA based on these results. Forty-two patients have already been entered into the most advanced phase II trial, a 2:1 randomization of FANG™ vaccine in "frontline" stage IIIc ovarian cancer patients with no evidence of disease following debulking surgery and standard adjuvant or sandwiched chemotherapy. Preliminary results of the non-blinded randomized trial are encouraging with a mean time to progression (disease recurrence or serial elevation of CA-125) from time of treatment of 346 days for FANG™ patients compared to 86 days for non-FANG™ treated patients. Planned accrual for this study is 100 patients. Based on preliminary results, we expect an opportunity to discuss a subsequent registration trial design (utilizing, for relevance to this transformative grant, a dual module vector and the bi-shRNAi platform) with the same patient population in the first quarter of 2013.

Earlier this year, we initiated a phase I safety trial of bi-shRNA-STMN1 (pbishRNA™ STMN1 LP). pbi-shRNA™ STMN1 LP (lipoplex) is a bi-functional expression vector targeting stathmin 1 (STMN1) oncogene encased in BIV (bilamellar invaginated vesicle) liposome. pbi-shRNA™ STMN1 LP is administered by a single intratumoral (IT) injection. STMN1 is a protein composed of 149 amino acids, often over-expressed in tumors and is involved in tubulin-microtubule compartmentalization, M-phase entrance and exit, and cell motility (Desai and Mitchison, 1997; Mistry *et al.*, 2005; Mitchison and Kirschner, 1984). A variety of target specific anti-stathmin effectors including ribozymes (Mistry *et al.*, 2001) and si-RNA (Alli *et al.*, 2007; Zhang *et al.*, 2006) have been used to silence stathmin *in vitro* as singlets (Alli *et al.*, 2007; Mistry *et al.*, 2001; Zhang *et al.*, 2006) and in combination with chemotherapeutic agents where additive to synergistic interactions have been demonstrated (Mistry and Atweh, 2006; Ngo *et al.*, 2007; Wang *et al.*, 2007). Both ribozyme and siRNA inhibition of stathmin mRNA result in an increase in G2/M phase cell population, an inhibition of clonogenicity, and a marked increase in apoptosis (Alli *et al.*, 2007; Iancu *et al.*, 2001; Zhang *et al.*, 2006). Patients with superficially accessible advanced cancer following prior therapies are study candidates with a starting intratumoral dose of 0.010 mg/kg of DNA. The primary objective is to determine the safety of intratumoral administration of pbi-shRNA™ STMN1 LP and the secondary objective to assess plasmid PK, detect cleavage product in injected tumor, and the third to determine STMN1 expression knockdown.

Future Directions

Insights from the application of information theory are beginning to be used to devise a strategic approach to cancer therapy (Brennan *et al.*, 2012). Clinical responses with single agents, despite targeting, are generally transitory, all too often followed by relapse or progression. The existence of intratumoral heterogeneity, signal pathway redundancy, pathway crosstalk, and vertical and horizontal feedback loops belie the effectiveness of single gene node targeting in the vast majority of solid tumors. Komarova and Wodarz (2005) devised a model of small molecule treatment for chronic myeloid leukemia based upon the disease volume at which resistance becomes a problem, the rate at which resistant mutants are generated, and the number of therapeutics employed. A minimum of three therapeutic agents was shown to be required to minimize the emergence of resistance. This assessment appears to hold for other biologic systems (Merl and Wessely, 2007). Using this strategy, a triple-target multiplex was chosen as an appropriate combination therapeutic to validate safety

and efficacy of multiple core pathway disruption. We are in a collaborative process of developing an integrated multiomic database to identify dominant signal pathways and gene hubs therein as the basis of triplex formulation which, we believe, represents the next generation of RNAi based cancer therapy. Ultimately, we envision a coordinated "one-two punch" attack against cancer comprising RNAi-based signaling pathway disruption and RNAi-based expanded "triad vaccine" (Figure 2). The rationale for this approach includes multiplex targeted therapy 1) to impair tumor growth to shift the tumor/T-cell activation kinetics ratio, 2) to induce apoptosis to enhance antigen presentation, and 3) to attenuate local/systemic immunosuppression along with immunotherapy, the effect of which is not limited to the duration of therapy and which can provide for long-lasting memory T-cell responses.

Conclusion

In summary, efficacy and preclinical safety have been demonstrated for several distinct RNAi technologies (i.e., siRNA, shRNA, and bi-shRNA). Both target specificity and anti-cancer activity have been shown in animal models. Thus far, systemic delivery to tumor targets in organs other than liver has been an intractable problem, but new platforms (e.g., BIV-LP) for safe and effective systemic delivery are being explored. RNAi technology has entered the clinic. The preliminary results from several phase I clinical trials are just now becoming available, but so far support the safety of these innovative cancer therapeutics. The demonstration of RNAi efficacy in cancer patients will hopefully emerge in the coming years.

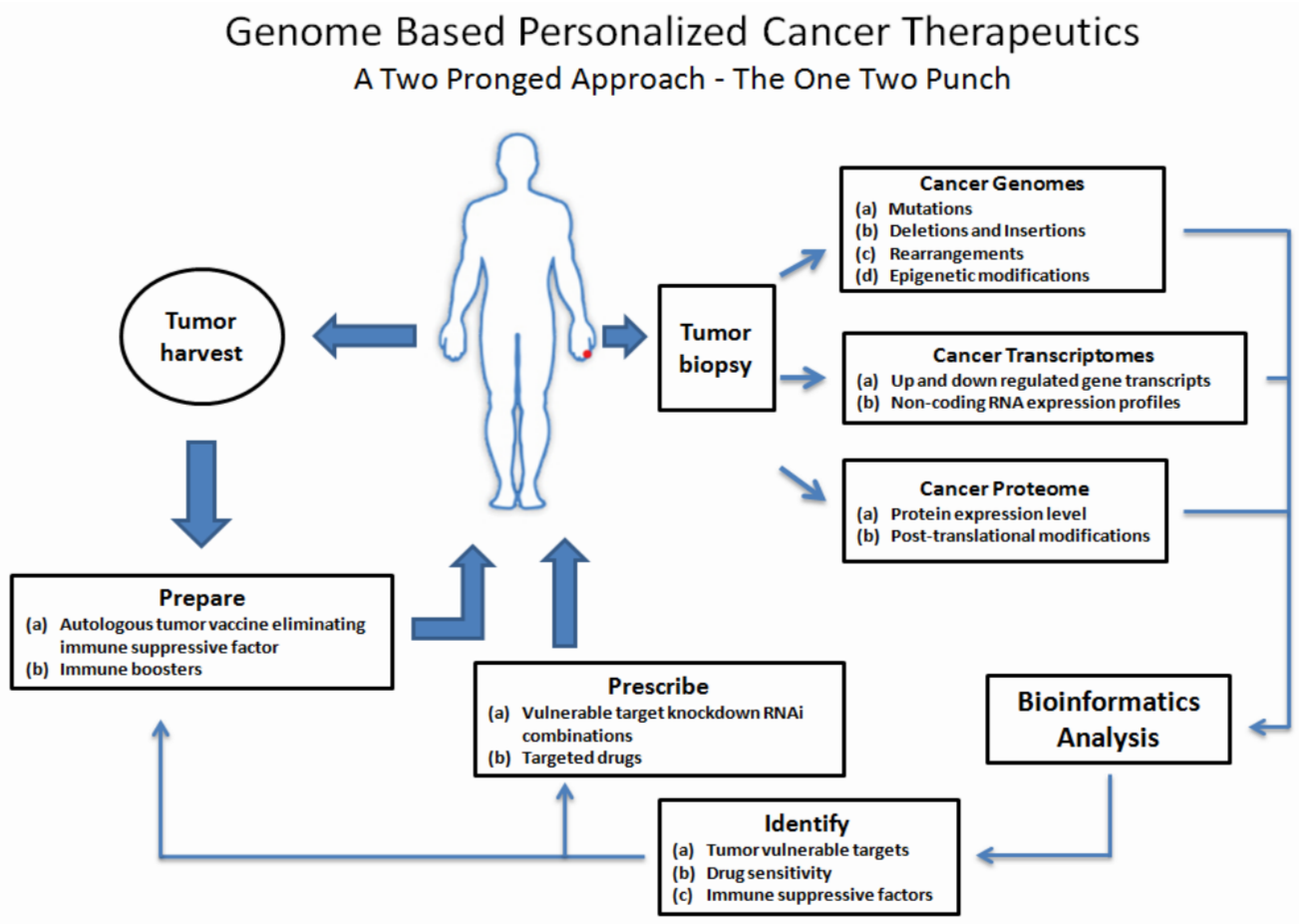

Figure 2. Genome Based Personalized Cancer Therapeutics, a Two Pronged Approach-The One Two Punch. Schematic illustration of the future of genome based personalized cancer therapeutic approach. The process starts with global DNA, RNA, and protein analysis of tumor samples from individual patient and compare the tumor tissue data to the normal tissue. Effective bioinformatics will be used to identify specific genetic and pathway abnormalities of each tumor and two pronged approach (targeted therapy and vaccine) will be prescribed for each individual patient for effective treatment.

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Disclosure

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