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Development of Novel Broad Spectrum Anticancer Small Molecule Peptidomimetics with Nanomolar Activity

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Introduction

Cancer is a major public health problem in the United States and throughout the world. It is currently the second leading cause of death in the United States and is expected to surpass heart diseases in the next few years to become the leading cause of death [1]. The estimated number of new cases of invasive cancer (all types) in the United States is 1,658,370 which is equivalent of more than 4,500 new cancer diagnoses each day. In addition, the estimated number of deaths from cancer in 2015 is 589,430 corresponding to about 1,600 deaths per day [1]. Though there has been a steady increase in survival for most cancers the death rate remains unacceptable and for certain cancers i.e. lung and pancreatic cancers the 5-year relative survival is currently 18% and 7%, respectively.

Traditional chemotherapy drugs act against all actively dividing cells (normal and cancerous cells) whereas targeted cancer therapies are drugs that interfere with specific molecular targets involved in cancer cell growth, progression and spread of cancer. Most targeted therapies are either small molecules or monoclonal antibodies. However, therapeutic strategies that target single molecular pathways eventually succumb to problems of intrinsic or acquired resistance due to extensive signaling "cross talk". Thus, combination targeted therapies are more attractive, as they synergistically inhibit multiple receptors. However, overlapping toxicities and pharmacological interactions limit patient compliance, feasibility and efficacy. Clearly, there is an urgent need to develop new first-line agents with enhanced efficacy and reduced toxicity.

We support the concept that the ideal drug maybe a broad spectrum drug whose efficacy is based not on the inhibition of a single target but rather a multi-targeted drug that affects several proteins or events that contribute to the etiology, pathogenesis and progression of diseases [2]. In addition, multi-pathway targeting is one of the strategies to overcome chemo-resistance.

To design novel anticancer drugs with unique structural properties we have taken an innovative and nontraditional approach where we combine pharmacophoric components to create new and highly potent small molecules with a simple three component "A-B-C" structure where each pharmacophore is known to have anticancer properties on its own or when incorporated as a component of an existing drug. Our multi-component "A-B-C" drugs can target simultaneously two or more different molecular targets or molecular mechanisms in a single entity which should reduce the likelihood of drug resistance.

Results and Discussion

Five stringent criteria were established before we would consider our compounds as drug leads:

1. Nanomolar broad spectrum activity against a large variety of human tumor cell lines including breast, colon, central nervous system (CNS), leukemia, melanoma, non-small cell lung (NSCL), ovarian, prostate and renal cancers;
2. The three component **"A-B-C"** molecules must be low molecular weight (500-700 Daltons) and consist of non-natural amino acids;
3. Easy and inexpensive to manufacture requiring only two chemical steps to join the three components together via two peptide bonds;
4. The compounds must be stable to proteolysis due to the use of non-natural amino acids;
5. The new structures must demonstrate synergistic activity over individual components.

In our new generation compounds we made very subtle changes in the structure of the A-component of GH101. GH501 differs from GH101 with a methyl group on the α-carbon and a fluorine atom on the phenyl group (Figure 1, where the A-component is Flurbiprofen) [3]. GH503 differs from GH101 with a methyl group on the α-carbon and the terminal phenyl group is replaced with a 4-carbon alkyl group (Figure 1, where the A-component is Ibuprofen). These subtle changes dramatically increased the activity of GH501 and GH503 compared to the starting prototype, GH101 by 3 to 5 fold in the 60 human tumor cell lines involving 9 cancer types tested by the NCI-60 tumor cell line screen and 5-10 fold against particular cell lines in each of the 9 cancer cell types. The average GI_{50} value for all 60 cell lines from the 9 cancer types was 498 nanomolar for GH501 and 498 nanomolar for GH503 compared to 1,350 nanomolar for our prototype, GH101. In addition, our new compounds GH501 and GH503 are also significantly more active than our first generation compound BKM570 [4] which had an average GI_{50} value of all 60 tumor cell lines in the NCI-60 cell line screen of 1,123 nanomolar. BKM570 remains an excellent candidate for the treatment of glioblastoma, the most aggressive brain tumor [5]. In conclusion, GH501 and GH503 are truly broad spectrum anticancer small molecules (Table 1). These results suggest that our new multi-component small molecules may have the potential to be effective in the treatment of all cancers.

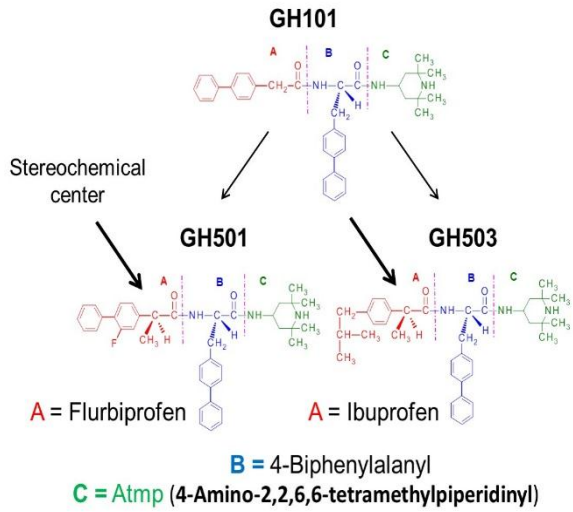

*Fig. 1. Two potent anticancer compounds (GH501 and GH503) were derived from our prototype, GH101 where the **A**-component in GH501 is Flurbiprofen and the **A**-component in GH503 is Ibuprofen. The **B**- and **C**- components are identical in both GH501 and GH503 and our starting prototype, GH101. The arrow shows the stereochemical center in the **A**-component.*

We have selected melanoma as our initial disease target before advancing to other cancer types since despite recent advancements in the treatment of melanoma, there is evidence that escape mechanisms arise and alternative therapies are urgently needed. An estimated 73,870 new cases of melanomas will be diagnosed in 2015 in the US with 9,940 deaths [1]. An important molecular feature of melanoma that impacts upon clinical drug development is that ~50% of all melanomas have activating mutations in the BRAF gene [6]. Of note, GH501 was effective against cell lines harboring the V600E BRAF mutation as well as wild-type BRAF suggesting that activity is independent of BRAF status (Table 2). The NCI-60 results were validated by Tentler and Eckhardt, at the University of Colorado, School of Medicine on 10 different melanoma cell lines with known BRAF status (Table 2).

To evaluate the antitumor *in vivo* efficacy of our lead compounds we will use a unique patient-derived tumor xenograft (PDTX) melanoma bank developed by Drs. Eckhardt and Tentler [7]. Because they are never cultured on plastic, PDTX models recapitulate the human tumor from which they were derived with respect to tumor architecture, stroma, mutational status, etc. and are thus considered superior models for drug efficacy studies [7]. As we have access to both BRAF mutant and BRAF WT PDTX models, we will assess the anticancer activity of GH501 and GH503 in both populations. BRAF mutant models will be compared with vemurafenib while BRAF WT will be compared with standard chemotherapy (dacarbazine). Additionally, the effects of GH501 and GH503 will be assessed in combination with vemurafenib or as a single agent in PDTX models of tumors that were derived from patients who developed resistance to vemurafenib clinically. To date, we have established over 30 individual patient tumor samples in nude mice, making our bank one of the largest in the country.

We have taken two approaches to identify the targets and the potential mechanism of action of our unique inhibitors. First, the Open Innovation Drug Discovery Program of Lilly suggested Histone H3 (Lys 27) methyltransferase (EZH2) as a potential target for our lead compounds, GH501 and GH503. EZH2 has recently emerged as an important and one of the most frequently mutated genes in

Table 1. Average GI_{50} values for compounds GH101, GH501 and GH503 against 9 cancer types.

Cancer Type	*Cell Lines Tested*	*Average GI_{50} (nanomolar)*		
		GH101	*GH501*	*GH503*
Breast	MCF7, MDA-MB-231/ATCC, HS 578T, BT-549, T-47D, MDA-MB-468	1,394	492	607
CNS	SF-268, SF-295, SF-539, SNB-19, SNB-75, U-251	1,315	398	411
Colon	COLO 205, HCC-2998, HCT-116, HCT-15, HT-29, KM12, SW-620	1,006	446	362
Leukemia	CCRF-CEM, HL-60(TB), K-562, MOLT-4, RPMI-8226, SR	592	241	225
Melanoma	LOX IMVI, MALME-3M, M14, MDA-MB-435, SK-MEL-2, SK-MEL-5, SK-MEL-28, UACC-62, UACC-257	1,753	598	592
Non-Small Cell Lung	A549/ATCC, EKVX, HOP-62, HOP-92, NCI-H226, NCI-H23, NCI-H322M, NCI-H460, NCI-H522	1,251	373	369
Ovarian	IGROV1, OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, NCI/ADR-RES, SK-OV-3	1,750	688	681
Prostate	PC-3, DU-145	1,600	360	415
Renal	786-0, A-498, ACHN, CAKI-1, RXF 393, SN12C, TK-10, UO-31	1,527	744	665
Average GI_{50} for 60 cell lines tested in the 9 cancer types		1,350	498	498

GI_{50} the concentration (nanomolar) that inhibits growth by 50%.

Table 2. GI_{50} values for 18 melanoma cell lines.

National Cancer Institute - NCI60		*University of Colorado*		
Melanoma Cell line	*GH501 GI_{50} (nanomolar)*	*Melanoma Cell line*	*GH501 GI_{50} (nanomolar)*	*BRAF*
LOX IMVI	150	HS294T	200	p.V600E
M14	260	HS695T	210	p.V600E
MDA-MB-435	267	AO4	250	WT
SK-MEL-5	377	A375	360	p.V600E
SK-MEL-28	428	MeWo	430	WT
UACC-62	882	GO4	690	p.V600E
MALME-3M	1200	1205LU	690	p.V600E
UACC-257	1220	HMCB	770	WT
		ME10538	920	p.V600E
		HS852T	1030	WT
Average GI_{50}	598		555	

Highlights are GI_{50} values less than 500 nanomolar.

melanoma, which has in turn sparked interest in targeting this molecule as a therapeutic option for this disease [8]. Second, to identify potential alternative mechanisms underlying the effectiveness of GH501 and GH503 on melanoma cell lines we will carry out metabolomics analyses. Metabolic changes closely mirror phenotypic alterations, making the metabolome a more reliable proxy than the genome, transcriptome or proteome for mechanistic studies. The main metabolic pathways related to energy and redox metabolism and how key metabolites are affected from incubation of melanoma cells with GH501 at 48 hours are shown in Figure 2.

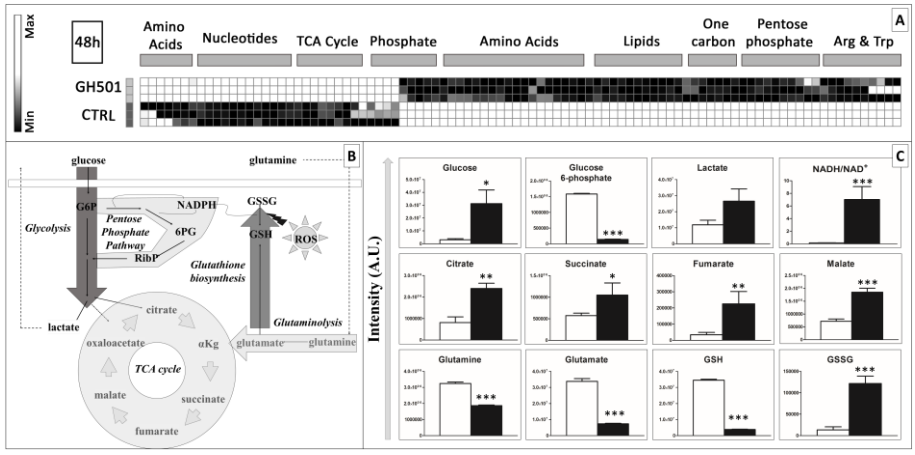

Fig. 2. Incubation of HS29T melanoma cells with GH501 for 48 h significantly altered metabolic profiles, as shown through the heat map in A (relative Z-score normalized fold changes are color coded; black=low; white=high levels). In B, we provide an overview of the main energy and redox metabolic pathways affected by the GH501 treatment (e.g. glycolysis, TCA cycle, Pentose Phosphate Pathway and glutaminolysis/glutathione homeostasis). In C, bar graphs are shown for key metabolites from these pathways for GH501-treated (black bars) and untreated cells (white). One to three asterisks indicate 95, 99 and 99.9% statistical confidence (paired T-test, n=3).

In summary, we have shown that our approach of creating a single multi-component "A-B-C" drug by rationally selecting pharmacophores based on general principles of our understanding of their anticancer activity, with no particular targets in mind and general screening of our compounds against the NCI-60 cell lines has provided new highly potent broad-spectrum anticancer drug candidates with nanomolar activity.

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