

Advanced Colorectal Cancer

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Eradication of Metastatic Colorectal Adenocarcinoma Resistant to Trastuzumab and Cetuximab After Treatment with Immunochemogene SEVINA-IV, a Stealth Nanoparticle Formulation Composed of Clamp PNA Against mRNA of sGC α 1/b1, Anti-MUC1 Chimeric Mab, and Vinorelbine Tartrate

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Objectives: Despite the high resectability rate, almost half of all patients with colorectal adenocarcinoma die from metastatic disease, primarily because of residual disease that is not apparent at the time of surgery. There is high unmet medical need in this setting due to the lack of treatment options. Soluble guanylyl cyclase (sGC) cytosolic enzyme, an obligatory heterodimer composed of α and β subunits, is overexpressed in colorectal adenocarcinoma, activating oncogenic pathways. Overexpression of MUC1 and bcl-2 causes immunoresistance and chemoresistance, respectively. We aimed to circumvent all these resistant factors, inducing programmed cell death in metastatic colorectal adenocarcinoma.

Methodology: We surgically obtained tumor cells from patients with stage IV immunochemoresistant colorectal adenocarcinoma, characterized by upregulation of MUC1, sGC heterodimer α 1 β 1, and bcl-2. We synthesized antisense clamp peptide nucleic acid (PNA) oligomers (DNA analogs), in which an N-C2-aminoethyl-glycine polyamide replaced the phosphate ribose ring backbone, and methylene carbonyl linker connected nucleobases to the central amine of N-C2-aminoethyl-glycine. The 6 mer homopyrimidine triplex [(PNA)₂/RNA] hybridized to the 5' end (Leader), and the 10 mer purine/pyrimidine duplex (PNA/RNA) hybridized to the 3'-end (Trailer) of the AUG start

codon region on the mRNA of sGC α 1/b1. The uncharged, and largely hydrophilic antisense clampPNA anti-sGC α 1/b1 was incorporated in the polar phase, and the lipophilic agent, vinorelbine tartrate molecules, was entrapped in the acyl-chains of the lipid phase, which was surrounded by the stealth/biocompatibility polymer layer and biologic recognition layer with linked chimeric monoclonal antibodies against MUC1 of the nanoparticle formulation, termed as SEVINA-IV. We used SEVINA-IV to treat xenograft animal models developed from colorectal adenocarcinoma cells obtained from the stage IV patients.

Results: Post-treatment, we observed that downregulated glycosylated transmembrane protein MUC1 blocked binding of tyrosine kinase inhibitors (TKI), such as cetuximab, and trastuzumab by inhibiting direct steric hindrance onto HER2/neu(c-erbB2), and EGFR via the MUC1 cytoplasmic tail. We also observed blocked binding of MUC1 onto IGF-1R. Subsequently, MUC1 phosphorylation was inhibited, blocking downstream signaling pathways, such as Ras/Raf/(Mek)Erk1/2/MAPK, PI3K/AKT, VEGF, and MMP-2. Antibody-dependent cellular cytotoxicity (ADCC) was induced. The clamp PNA hybridized to the leader and trailer region of the AUG start codon region on mRNA sGC, forming Watson-Crick double helices and steric hindrance of the translation machinery, inhibiting gene expression of sGC α 1/b1 after assembly inhibition of the 80S ribosome initiation complex. Inhibited translation of nitric oxide (NO) receptor sGC α 1/b1 resulted in complete loss of NO activity leading to a 400-fold reduction in formation of cGMP from GTP, inactivating protein kinase, ion-channels, and phosphodiesterases. Inhibition of NO blocked S-nitrosylation of cysteine-118 leading to inactivation of Ras, and cGMP-dependent downregulation of COX-2, and NF- κ B transcriptional activity inducing p53 independent apoptosis or type I PCD. Suppression of overexpressed oncogene Ras (mutant in codon 12) downstream mediators, such as Raf/MEK/ERK1/2, was observed. Inhibition of B-Raf downregulated VEGFR-1-2-3, PDGFR-b, FIR-3, and FGFR-1, and upregulated p21CIP1, p16INK4a, and p27KIP1. Downregulation of ERK1/2 blocked VEGF-induced endothelial cell proliferation, after inhibition of endothelial NO synthase, blocking angiogenesis, lymphangiogenesis, perivascular cell recruitment, and stabilization of blood and lymphatic vessels. The tumor suppressor genes RIN, TSC2, and PTEN were

upregulated, and PI3K/AKT/mTOR pathway was blocked, inhibiting binding to Ras upstream mediators, such as the HER2 family receptors, IGFR, PDGFR, and EGFR. Inhibition of soluble guanylyl-cyclase/guanosine 3',5'-cyclic monophosphate signaling blocked hyperphosphorylation of pRb, leading to its upregulation, and inactivated mTOR and its downstream effectors p70 S6K and eIF4E of the translational machinery, leading to the reduced rate of inhibitor of apoptosis protein (IAP) survivin, and two integral cell cycle transit proteins, cyclin D1 and ODC. Inhibition of eIF4E led to strong autocrine/paracrine downregulation, inducing apoptosis after inactivation of cell cycle promoters, such as Ras, PGF, CDK2, CDK4, CDC25B, and SKP2. There was inhibition of HIF-1 downstream genes and tumor-associated inflammatory cells (macrophages), blocking release of prostaglandin E2(PGE2) derived from COX-2 inhibition of NO production due to inactivation of isoforms of NO synthase (NOS), such as inducible NO synthase (iNOS), and endothelial NO synthase (eNOS), which in turn blocked interaction with different molecular targets, such as superoxide anion and protein macromolecules. This led to inhibition of colorectal adenocarcinoma progression, proliferation via the E2F1 pathway, translation, total protein synthesis, and phosphorylation of apoptotic tumor suppressor genes leading to their upregulation. Finally, colorectal adenocarcinoma cells are characterized by upregulation of antiapoptotic oncogene bcl-2, which binds to tumor suppressor gene beclin-1, inhibiting its release, and subsequent induction of caspase-independent type II PCD or autophagy. Vinorelbine-tartrate, by phosphorylating bcl-2, led to its inactivation, depolymerizing cytoskeleton microtubules, which blocked cell cycle at G2/M phase. Release of tumor suppressor gene beclin-1 and upregulation of BIM induced type II PCD or autophagy. DNA synthesis and metabolic activity of tumor cells was inhibited according to BrdU, and MTT tests, respectively. Transmission electron microscopy (TEM) exhibited three types of programmed cell death, such as type I PCD/apoptosis, type II PCD/autophagy, and type III PCD/necrosis.

Conclusion: Administration of SEVINA-IV eradicated metastatic colorectal adenocarcinoma after phosphorylation of bcl-2, inhibition of MUC1, translation of sGCa1/b1, and downstream signaling pathways, circumventing resistance to trastuzumab and cetuximab.