Are we there yet? An update on oligonucleotide drug development

Abstract Oligonucleotide therapeutics are a new class of drugs with only three commercialized drugs to date. In spite of this limited commercial success, the oligonucleotide field continues to grow rapidly. Many drugs have advanced to late clinical phases. A variety of drug delivery strategies have proven effective, and improvements continue to be made in the manufacturing technologies used to produce oligonucleotides. Growth in the oligonucleotide field is fueled by the renewed interest of large pharmaceutical companies and the emergence of startup companies with new intellectual property. The oligonucleotide field appears to be poised for success.

KEYWORDS: Oligonucleotide therapeutics; solid phase oligonucleotide synthesis; drug development; drug delivery.

INTRODUCTION

Oligonucleotide therapeutics have tantalized drug developers with the promise of rational drug design, lower drug development costs, and the ability to reach targets that conventional small molecule drugs cannot. Three oligonucleotide drugs have been approved by regulators, but oligonucleotides have not yet fully delivered upon their elusive promise. Large pharmaceutical companies have entered, exited, and then re-entered the oligonucleotide field in dramatic fashion. In 2012, Dr. Art Krieg, co-founder of Coley Pharmaceutical Group and now CEO of Checkmate Pharmaceuticals, summarized the evolving perspective of pharmaceutical companies towards oligonucleotides. Dr. Krieg stated that the first phase is an irrational exuberance that is followed by excessive skepticism when problems arise. Excessive skepticism is then followed by resurging enthusiasm and reinvestment as products are developed (1). Unfortunately, the period of excessive skepticism towards oligonucleotides also coincided with the economic turmoil of the Great Recession resulting in several very bleak years for the field. Venture capital was difficult to obtain, and frequently small biotech companies were forced to scale back or delay clinical trials due to lack of funding. The last several years have been marked by a resurging enthusiasm in oligonucleotide therapeutics. Numerous clinical candidates have advanced, and several drugs appear poised for regulatory approvals. Big pharmaceutical companies seem to once again be investing in oligonucleotides through licensing deals, partnerships, and acquisitions. An example of this shift in enthusiasm is the Roche acquisition of Danish company Santaris Pharma for $250M upfront and potential payout of $200M based on achievement of certain predetermined milestones (2). This announcement came in August 2014, nearly four years after Roche announced it was restructuring and discontinuing its RNAi research center in Kulmbach Germany. Raw material suppliers, equipment manufacturers, and contract manufacturing organizations have all responded to the renewed interest in oligonucleotides, and they continue to innovate in order to meet the changing needs of the market.

OVERVIEW OF CLINICAL CANDIDATES AND TRENDS

The number of oligonucleotides currently in clinical trials is still small relative to other therapeutic classes. According to Nitto Denko Avecia internal research, just 116 oligonucleotides were in active clinical trials as of August 2015. In spite of the small numbers, the drugs in clinical trials continue to advance. From 2007 to 2011, the biggest increase in clinical movement came from oligonucleotides advancing from preclinical development into Phase 1 clinical trials. Those numbers shifted in recent years. From 2012 to 2015, the biggest increase was in the oligonucleotides moving from Phase 1 to Phase 2. By mid 2015, twice as many oligo therapeutics were in Phase 2 as were in Phase 1 demonstrating that the oligonucleotide clinical pipeline is further maturing. Oligonucleotides are comprised of many different classes including antisense, RNAi (siRNA and miRNA), immunostimulatory, aptamers, and decoys. Antisense drugs still represent the largest class of oligonucleotides in the clinic at nearly 40%, but siRNA has narrowed the gap and now accounts for nearly 30% of oligos in clinical trials. In 2015, the number of siRNA pre-clinical programs slightly exceeded the number of antisense pre-clinical programs, so it is likely that more RNAi drugs will enter Phase 1 in the years to come. Predicting which oligonucleotide drug will be next to gain regulatory approval is difficult. Many thought that BioMarin’s exon skipping oligonucleotide, drisapersen, used to treat Duchene’s Muscular Dystrophy (DMD) would garner the next approval. The drug had been developed by the Dutch biotech company, Prosensa before it was acquired by BioMarin, a pharmaceutical company specializing in rare diseases. Observers were cautiously optimistic that this orphan drug would be approved even though GSK, who had partnered with Prosensa previously, opted to discontinue their collaboration with Prosensa and return the rights to drisapersen back to Prosensa in early 2014 (3). In January of this year, the US FDA declined approval of the drug citing that the standard of substantial evidence of effectiveness had not been met (4). BioMarin is now left to see if there is a path forward for their drug. Sarepta Therapeutics is developing Eteplersen, another drug for the treatment of DMD. Eteplersen is a phosphorodiamidate morpholino oligomer (PMO). The FDA extended the PDUFA date to May 26, 2016, so DMD patients will have to wait to learn of FDA’s decision (5). The next commercial oligonucleotide product might...
come from the vaccine world, Dynavax’s adult hepatitis B vaccine Heplisav-B™ combines a hepatitis surface antigen with a proprietary immunostimulatory oligonucleotide that acts as a TLR 9 agonist. In a press release announcing favorable topline results from the latest Phase 3 clinical trial, Dynavax CEO Eddie Gray stated, “We are delighted to report these topline results from HBV-23 and confirm our intention to resubmit the HEPLISAV-B BLA by the end of March. These results support our belief that HEPLISAV-B, if approved, could offer benefits to adults at risk for hepatitis B, particularly given that these significant differences in seroprotection were demonstrated in a controlled setting, where compliance is optimized” (6).

Oligonucleotide juggernauts, Alnylam Pharmaceuticals and Ionis Pharmaceuticals (formerly Isis Pharmaceuticals), both have drugs in Phase 3 clinical trials for the TTR-Mediated Amyloidosis clinical syndromes familial amyloidotic polyneuropathy (FAP) and familial amyloidotic cardiomyopathy (FAC). Both conditions represent serious unmet medical needs, and patients and clinicians wait with anticipation to see if they might soon have multiple treatment options for these conditions.

Other oligo drugs in late clinical phases show a surprising amount of diversity in their indications and formulations. Ophthotech initiated a Phase 3 clinical trial with their lead candidate Fovista® used to treat wet age-related macular degeneration (AMD). The drug is an aptamer that binds to platelet derived growth factor (PDGF) and is administered via intravitreal injection. Anges’ NF- B decoy oligo for the treatment of atopic dermatitis drug is in ongoing Phase 3 trials in Japan. The oligo is formulated as an ointment and applied topically. InDex Pharmaceuticals is developing Kappaproct®, a 19 mer DNA that acts as a TLR-9 agonist, for the treatment of ulcerative colitis (UC). The drug is administered locally to the colonic mucosa and has shown statistically significant improvement of symptomatic remission (blood in stool, number of stools) and registration remission (clinical remission with concurrent endoscopic remission) as well as rate of colectomy (7). Gene Signal’s drug, Aganersin, exhibited positive Phase 3 data (8). The antisense oligonucleotide is used to treat corneal neovascularization in graft patients and is administered topically with eye drops. Atlantic Healthcare announced initiation of a Phase 3 clinical trial for their antisense oligo drug, alicafosren, used to treat inflammatory bowel disease pouchitis (9). The drug was developed by Ionis Pharmaceuticals and is an antisense inhibitor of ICAM-1. Alicafosren is formulated as an enema that patients can administer at home rather than in a clinical setting. Biogen has also partnered with Ionis Pharmaceuticals on multiple clinical candidates including a Phase 3 drug for infants and children with spinal muscular atrophy. The pipeline of oligonucleotide drugs in late clinical stages has never been stronger.

**DRUG DELIVERY**

Researchers have struggled to find effective methods for systemic delivery of oligonucleotides. Nearly all of the drugs previously noted in late stage clinical trials are delivered locally. Liposomal based drug delivery systems have successfully been used to deliver siRNA and antisense oligonucleotides to cells (10). Individual companies have developed intellectual property around specific liposomal delivery systems. Mirna Therapeutics holds the exclusive license for SMARTICLES®, the liposomal delivery technology developed by Novosom AG and licensed from Marina Biotech, Inc. Mirna has used SMARTICLES® for delivery of MRX34, the first microRNA mimic in a Phase 1 clinical trial (11). Tekmira Pharmaceuticals (now Arbutus Biopharma) developed proprietary lipid nanoparticle technology (LNP), which they utilized for systemic delivery of siRNA in clinical trials. The technology was licensed to others including Dicerna Pharmaceuticals and Alnylam Therapeutics for systemic delivery of siRNA drugs in clinical trials. More recently, researchers have found that conjugation of siRNA to N-acetylgalactosamine (GalNac) allows for subcutaneous administration at therapeutically relevant doses and dose volumes of 1 mL or less [12]. GalNac can be conjugated to oligonucleotides at the 3’ or 5’ end, and the conjugation chemistry is compatible with conventional synthesis, cleavage, deprotection, and purification conditions.

Celgene Corporation is developing an antisense drug, GED-0301, for the treatment of Crohn’s disease. The drug is delivered as a solid dosage form designed to release the drug in the far end of the small intestine and near end of the colon. A Phase 3 clinical trial is scheduled to be initiated this year [13], and this would mark the first time an orally administered oligonucleotide advanced to Phase 3.

**MANUFACTURING THERAPEUTIC OLIGONUCLEOTIDES**

Few oligonucleotide drug sponsors have their own internal cGMP manufacturing capabilities, and over half of the oligonucleotides in clinical development are manufactured at a contract manufacturing organization (CMO). As more oligonucleotides move towards regulatory approval, the demand for manufacturing capacity will increase forcing CMOs to add capacity and look for ways to improve efficiencies through innovation. German CMO BioSpring announced in 2014 the addition of a midscale synthesizer to increase manufacturing capacity. Results were presented from the first synthesis with GE Healthcare’s next generation of OligoProcess Synthesizer (14). Nitto Denko Avecia, the market leader in contract manufacturing of oligonucleotides, has implemented the use of thin film evaporation to concentrate product solutions prior to lyophilization allowing for larger final batch sizes [15]. Some drug sponsors are choosing to add internal manufacturing capacity rather than relying on CMOs. Alnylam Pharmaceuticals purchased 12 acres of land in southeastern Massachusetts where they will build a $200M drug manufacturing plant. Alnylam plans to break ground in 2016 and complete construction by 2018 [16]. Recent improvements in polymeric solid supports have allowed manufacturers to significantly increase
synthesis throughout. In 2010, InnovaLife Sciences, Inc. and Nitto Denko Corporation announced the launch of Nitto Phase® HL – High Loaded Solid Support for oligonucleotide synthesis. The support can be loaded up to 400 µmol/g for DNA and 250 µmol/g for RNA (17). Previous polymeric supports were available at 200 µmol/g. With higher loaded supports, manufacturers can produce more oligonucleotide per unit operation without changing equipment. GE Healthcare also released their fifth generation of polymeric support, Primer Support 5G, with loading as high as 350 µmol/g for DNA and 300 µmol/g for RNA (18). Oligonucleotide manufacturers now have a choice of high loaded support.

Recent innovations in phosphoramidites, the building blocks of oligonucleotides, have been mostly focused on RNA chemistry. RNA chemistry is currently dominated by the use of TBDMS protected phosphoramidites, but other novel building blocks are now commercially available. The 2′-thiocarbamoyl (TC) protected amidites developed at Agilent Technologies (19) and 2′-pivaloyloxymethyl (PivOM) developed by the Diebart group in France (20) provide the benefit of not requiring a separate processing step for removal of the 2′-protecting groups. Production time can be reduced since base deprotection and 2′-protecting group removal can be done in one single step. ChemGenes Corporation developed a novel building block by employing conventional base and ribose protecting groups and switching the direction of oligonucleotide synthesis from 3′-5′ to 5′-3′. This is achieved by placing DMTr protecting group at the 3′-position instead of the 5′-position and placing the phosphoramidite moiety at the 3′-terminus. This approach allows for efficient 3′-ligand attachment significantly improving the RNA synthesis overall (21). Building blocks that create a neutral oligonucleotide backbone are attractive since oligonucleotides with neutral backbone modifications tend to have high nuclease resistance and high cell permeability.

Triphos Therapeutics, Inc. developed BMEG amidites that incorporate a novel neutral modification that serves as phosphate bio-labile protecting group, which reversibly masks the oligonucleotide’s negative charge (22). Having alternative, novel building blocks available is important as researchers look to discover the next generation of oligonucleotide therapeutics.

Japanese based Ajinomoto reported having a solution phase process that utilizes their proprietary support, AJIPHASE®, to produce hundreds of grams of oligonucleotides (23). The technology is still new and needs to be further demonstrated, but it could provide an attractive alternative for companies that need large quantities of oligonucleotides that can be produced in a standard chemical plant without investment in large scale oligonucleotide synthesizers. Solution phase processes tend to require significant amounts of upfront process development work, while conventional solid phase synthesis does not. Drug developers will need to decide at what point in the development cycle they want to invest in developing a solution phase based process. However, the prospect of having a viable solution phase process for large scale manufacturing is appealing, and many will watch to see how the technology develops.

NEW TECHNOLOGIES WILL PRODUCE NEW CLINICAL CANDIDATES

New start-up companies with new technologies and rich patent portfolios are aiming to advance entirely new classes of oligo therapeutics. Seattle based Halo-Bio develops multivalent RNA molecules (MV-RNA) for gene targeting. MV-RNA utilizes the same RISC pathway as siRNA, but expands gene targeting, eliminates, sense-strand off-target effects, and introduces the first “junction containing” structures to RNAi. The MV-RNA are composed of three guide strands that each align to a potential target site (24). WAVE Life Sciences is developing stereopure nucleic acid therapeutics. Most oligonucleotide therapeutics contain multiple phosphorothioate bonds to improve stability against nuclease degradation. Each phosphorothioate bond creates a chiral center. Oligonucleotide drug developers have long known that their drugs consisted of a mixture of stereoisomers, but the mixtures were far too complicated to purify or analyze. The scientists at WAVE believe by controlling stereochemistry, they can produce drugs that will quickly become the best in their class. The company recently announced that six clinical programs will be delivered by 2018, and the first two INDs for Huntington’s Disease are targeted for 2016 (25). Moderna Therapeutics is not a traditional oligonucleotide company since their drug candidates are not chemically synthesized using standard phosphoramidite chemistry. However, their mRNA platform is poised to deliver multiple novel clinical candidates. The company announced that they were transitioning to a clinical stage company as their first Phase 1 study was underway in Europe (26), and the company expanded from 192 people to 326 people in 2015. These three companies are just a few of the organizations working to innovate and deliver the next generation of oligonucleotide therapeutics.

CONCLUSION

The oligonucleotide field is about to reach a critical turning point. Never before have so many oligo therapeutics been in Phase 3 clinical trials. These drugs span a variety of oligonucleotide classes, indications, and routes of administration. Within the next 5 years, regulatory approval of multiple oligonucleotide therapeutics seems likely. An increase in venture capital following the Great Recession and the renewed interest by big pharma has injected renewed energy into the field. New startup companies with novel technologies will likely continue to propel the field forward and fuel the development pipeline with new therapeutic options. One is left with the feeling that oligonucleotide drugs are now closer than ever to delivering upon their promise and impacting the lives of patients.

ACKNOWLEDGEMENTS

The author thanks Andrei Laikhter of Chemgenes Corporation, Wilmington, Massachusetts for summarizing the developments in RNA phosphoramidite chemistry.
Many modified oligonucleotides have been used as tools in the study of gene regulation and in different hybridization-based assays. In particular, modified oligonucleotides have been used in applications such as antisense gene regulation, drug discovery. In particular, modified oligonucleotides have been used as tools in the study of gene regulation and in different hybridization-based assays. When one is designing oligonucleotide molecules for antisense studies, there are three major considerations. The most commonly used antisense oligonucleotide molecule to nuclease activity. Finally, a characteristic important for in vivo study is the ability of the molecule to undergo transmembrane transport (uptake) into the cell. The most commonly used antisense oligonucleotide composition is a gapmer made of chimeric oligonucleotide.

REFERENCES

17. Kinovate product literature
18. GE Healthcare Primer Support 5G product literature
22. S. Petersen et al., US Patent 8691971.

ANDREI LAIKHTER
Chief Scientific Officer, Chemgenes Corporation

Recent achievements in therapeutic oligonucleotide chemistry

KEYWORDS: Antisense, targeting mRNA, RNAi Technology, exon-skipping therapy.

In recent times both basic and applied molecular biology studies have made extensive use of modified oligonucleotides as tools in the study of gene regulation and drug discovery. In particular, modified oligonucleotides have been used in applications such as antisense gene regulation and in different hybridization-based assays.

ANTISENSE AND EXON SKIPPING TECHNOLOGIES

Many modified oligonucleotides have been used as antisense molecules, targeting mRNA for the study of gene regulation. These DNAs or their analogues can hybridize to the complementary region of a corresponding DNA or RNA and affect gene expression. For antisense molecules that target mRNA, gene expression is suppressed by Rnase H catalyzed cleavage of the bound mRNA of the duplex. When one is designing oligonucleotide molecules for antisense studies, there are three major considerations. The affinity and stability of heteroduplexes of mRNA with DNA or its modified analog should be taken into account. Furthermore, one must consider the vulnerability of the molecule to nuclease activity. Finally, a characteristic important for in vivo study is the ability of the molecule to undergo transmembrane transport (uptake) into the cell. The most commonly used antisense oligonucleotide composition is a gapmer made of chimeric oligonucleotide.
21-24 nucleotides long and the terminuses 3-4 nucleotides with the ribose moiety having predominant 3'-endo configuration that enhancing hybridization property of the chimeric oligonucleotide, the middle (gap) is usually phosphorothioated DNA that is from one hand more nuclease resistant compared to natural phosphate diester DNA and from other hand enhances RNA cleavage in presence of RNase H, when it hybridized to the target mRNA molecule. The most common approach is to stabilize 3'-endo sugar conformation using 2'-O-Me, 2'-F and 2'-O-methoxymethyl (2'-MOE) chemistries (1, 2). Thus the conformationally restricted structures locked in 3'-endo structure (LNA) even more attractive and it has been evaluated in antisense study (3-6).

Recently the antisense approach resulted in successful development of hypercholesterolemia drug Kynamro ( mipomersen sodium) by ISIS Pharmaceuticals has been commercialized for treatment of hypercholesterolemia (13). The neutral oligonucleotide backbone modifications have been developed at ChemGenes Corporation by employing conventional base and ribose protecting groups but switching direction of oligonucleotide synthesis from 5'-3' to 3'-5' or reversing it by placing DMTr protecting group to 5'-3' position instead of 5'-position and placing phosphoramidite moiety at 3'-terminus. That approach enables several new options especially for efficient 3'-ligand attachment using corresponding amidites, significantly improves RNA synthesis overall (13) and apparently pushing the limitation for ultra-mer synthesis to over 200 nt.

The neutral oligonucleotide backbone modifications always have been in the focus of oligonucleotide therapeutic study. That type of oligonucleotide modifications has been attractive due to high nuclease resistance and high cell permeability. BMEG is the novel neutral modification that serves as phosphate bio-degradable protecting group developed by Scott Petersen (14) at Triphas Therapeutics, Inc.

**RNAi TECHNOLOGY**

The short interfering RNA (siRNA) are naturally accruing double stranded RNA molecules that bind RNA-induced silencing complex or RISC and subsequently recognizes and cleaves the complement mRNA strand that is resulting in gene silencing. Since chemically modified RNA molecules have been used and evaluated in anti-sense technology (1-6), the same type of modifications such as 2'-O-Me, 2'-MOE and 2'-F have been successfully employed in siRNA therapeutic technology (7-8). The chemically modified siRNA molecules demonstrated much higher nuclease resistance compared to natural RNA and good affinity to RISC complex that leads to an efficient gene silencing (9). The therapeutic success of RNAi technology represented in over twenty clinical trials leading by Alnylam Pharmaceuticals, Tekmira Pharmaceuticals and Quark Pharmaceuticals currently in stages I and II (10) targeting different types of cancer, Ebola virus and hypercholesterolemia. This significant break through in pharmaceutical RNAi technology is supported by new developments in RNA chemistry as well. During last decade in addition to two conventional RNA synthesis chemistries such as TGM and TBDMS three new types of RNA building blocks have been developed and became commercially available for ribo oligonucleotide synthesis. Two of them 2'-thiocarbamoyl (TC) developed at Agilent Technologies (11) and 2'-pivaloyloxymethyl (PivOM) developed by Diebert group in France (12). These chemistries don’t require separate processing step for 2'-protecting groups removal from the RNA oligomer therefore both base deprotection and 2'-protecting group removal can be done in one single step and that makes these chemistries suitable for high thoughput (HTP) synthesis platforms. Another novel building blocks

have been developed at ChemGenes Corporation by employing conventional base and ribose protecting groups but switching direction of oligonucleotide synthesis from 3'-5' to 5'-3' or reversing it by placing DMTr protecting group to 3'-position instead of 5'-position and placing phosphoramidite moiety at 3'-terminus. That approach enables several new options especially for efficient 3'-ligand attachment using corresponding amidites, significantly improves RNA synthesis overall (13) and apparently pushing the limitation for ultra-mer synthesis to over 200 nt.

The neutral oligonucleotide backbone modifications always have been in the focus of oligonucleotide therapeutic study. That type of oligonucleotide modifications has been attractive due to high nuclease resistance and high cell permeability. BMEG is the novel neutral modification that serves as phosphate bio-degradable protecting group developed by Scott Petersen (14) at Triphas Therapeutics, Inc.

**MICRO-RNAS AND LONG NON-CODING RNAs**

Micro RNAs or miRNA are single stranded hairpin RNA molecules derived from long non-coding RNA and play an important role in gene regulation (15). miRNA employs the same RISC machinery as siRNA. That fact makes both miRNAs and long non-coding RNAs an important biomarkers in numerous cases of the genetic abnormalities (16).
The Clinical development of the toll-like receptor 9 agonist DIMS0150 in chronic active ulcerative colitis patients

KEYWORDS: Toll-like receptors, ulcerative colitis, therapy, oligonucleotide.

ULCERATIVE COLITIS

Ulcerative colitis (UC) is an immune-mediated condition characterized by a continuous mucosal inflammation of the colon, which might be caused from the dysregulated balance between commensal enteric flora and the gut-associated immune system. A proportion of patients do not respond to available therapies becoming treatment refractory and requiring surgical intervention and therefore novel treatment strategies are needed (1-3).

One of the ways that host discerns foreign from self-antigen is through pattern recognition receptors (PRRs), which recognize specific molecular patterns of pathogens. One group of PRRs consists of Toll-like receptors (TLRs) with variable specificities for sensing microbial products. One of these receptors, TLR-9, recognizes exclusively bacterial DNA and has over the years received growing interest as a potential target for therapeutic intervention in the treatment of UC. It could be shown that TLR-9 activation prevents development of mucosal inflammation and promotes wound healing in several models of experimental colitis (4-6).

DIMS0150

DIMS0150 is a fully synthetic DNA based 19 mer oligodeoxyribonucleotide that acts as a TLR-9 agonist, developed by our company. This DNA sequence elicits its immunomodulatory effects through specific target cells, i.e., antigen presenting cells such as dendritic cells and B cells, as well as intestinal epithelial cells. A large number of in vitro and in vivo studies have shown that DIMS0150 exerts immunomodulatory as well as anti-inflammatory effects. The immunomodulating effects of DIMS0150 are accomplished through the induction of e.g. interferons type I and Interleukin 10 (IL-10) from these target cells.

DIMS0150 is currently under clinical evaluation in the indication ulcerative colitis (UC) for its potential ability to induce clinical remission in UC patients who are chronic active treatment refractory. DIMS0150 has been assessed in a total of four clinical trials and under named patient use, comprising a total of 249 patients that have received at least one dose. Local administration of DIMS0150 onto the colonic mucosa has shown positive effects in chronic active ulcerative colitis patients with respect to efficacy and safety. In the latest clinical study the efficacy of DIMS0150 was evaluated in a randomized, double blind, placebo-controlled, multicentre, pan-european trial (the “COLLECT” study) in 131 patients with moderate to severe active ulcerative. Patients were randomly assigned to receive two single doses of DIMS0150 (30 mg) or placebo (in a 2:1 ratio) administered topically to the inflamed mucosa at baseline and after 4 weeks. Already at week 4 significantly more DIMS0150 treated patients achieved symptomatic remission compared to placebo. Furthermore, the proportion of patients in clinical remission with mucosal healing at week 4 was also higher in the DIMS0150 group versus the placebo group. At later time points e.g. at week 8 or 12 sustained efficacy was observed. In this clinical study a total of 13.8% SAEs were reported across both treatment groups with 18.6% of patients in the placebo group and 11.5% patients in the Kapparot group reporting serious AEs, supporting the previously observed safety profile of DIMS0150.

CONCLUSION

Therapy with the topically applied TLR-9 agonist DIMS0150 is a promising and well-tolerated novel therapeutic option for treatment refractory, chronic active UC patients warranting further clinical trials.

REFERENCES