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Insights into the Chemical Discovery of Remifentanil

Paul L. Feldman, Ph.D.

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Design, Synthesis, and Pharmacological Evaluation of Ultrashort- to Long-acting Opioid Analgetics. By Feldman PL, James MK, Brackeen MF, Bilotta JM, Schuster SV, Lahey AP, Lutz MW, Johnson MR, Leighton HJ. J Med Chem 1991; 34:2202-8. Copyright 1991 American Chemical Society. Reprinted with permission.

Abstract: In an effort to discover a potent ultrashort-acting μ-opioid analgetic that is capable of metabolizing to an inactive species independent of hepatic function, several classes of 4-anilidopiperidine analgetics were synthesized and evaluated. One series of compounds displayed potent μ-opioid agonist activity with a high degree of analgesic efficacy and an ultrashort to long duration of action. These analgetics, 4-(methoxycarbonyl)-4-[1-oxopropyl)phenylamino]-1-piperidinepropanoic acid alkyl esters, were evaluated *in vitro* in the guinea pig ileum for μ-opioid activity, *in vivo* in the rat tail withdrawal assay for analgesic efficacy and duration of action, and *in vitro* in human whole blood for their ability to be metabolized in blood. Compounds in this series were all shown to be potent μ agonists *in vitro,* but depending upon the alkyl ester substitution, the potency and duration of action *in vivo* varied substantially. The discrepancies between the *in vitro* and *in vivo* activities and variations in duration of action are probably due to different rates of ester hydrolysis by blood esterase(s). The [structure–activity relationships] with respect to analgesic activity and duration of action as a function of the various esters synthesized is discussed. It was also demonstrated that the duration of action for the ultrashort-acting analgetic, 8, does not change upon prolonged infusion or administration of multiple bolus injections.

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In 1987 the armamentarium of μ-opioid "short-acting" analgesics for anesthesiologists consisted of fentanyl, discovered by Paul Janssen, M.D., founder of Janssen Pharmaceutica in Beerse, Belgium, and approved for use in 1968, along with sufentanil and alfentanil, both discovered by Janssen *et al.* and approved for clinical use in the mid-1980s (fig. 1). Fentanyl's introduction supplanted the use of morphine and other older opioid-based analgesics used during surgical procedures, in part due to problems with histamine release and their pharmacokinetic properties, which often led to prolonged respiratory depression. Fentanyl's profile was a significant advance relative to these classic opioids and ushered in a new and superior class of opioid analgesics for use in surgical settings. Sufentanil was marketed as a more potent analgesic than fentanyl with a slightly faster onset of action and alfentanil was promoted based on having the most rapid onset of action coupled with the shortest duration of action among these three next-generation opioid analgesics. These drugs, at the time, were primarily used as adjuncts to anesthetics and to relieve pain during surgery. Research continued to further improve their profiles.[1]

Paul L. Feldman, Ph.D.

In late 1987, I joined Glaxo, Inc. (Research Triangle Park, North Carolina) as a neophyte medicinal chemist after just completing my doctoral work in synthetic organic chemistry at the University of California, Berkeley, California. Glaxo had just commenced its discovery efforts in the United States in Research Triangle Park, North Carolina, and I was part of the first wave of medicinal chemists hired. The new medicinal chemistry recruits knew a lot about organic chemistry, but we lacked knowledge and experience in medicinal chemistry and thus, were placed in the capable managerial and medicinal chemistry-experienced hands of M. Ross Johnson, Ph.D., who had been recruited by Glaxo from Pfizer Inc. in Groton, Connecticut, to lead, build, and nurture a medicinal chemistry department.

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I was asked to lead the medicinal chemistry effort to discover an ultrashort-acting μ-opioid agonist.[2] Jeffrey Leighton, Ph.D., who headed pharmacology at Glaxo and had recently joined from Burroughs Wellcome Company in Research Triangle Park, North Carolina, conceived of the idea to discover an opioid analgesic within the chemical structural class of fentanyl and render its pharmacology ultrashort-acting by making it susceptible to esterases to inactivate its μ-opioid activity. The rationale for discovering such an agent was that the current opioid analgesics in clinical practice were too long-acting. They relied on hepatic metabolism for clearance, and the drugs accumulated during longer surgical procedures, making patient recovery protracted. Offering an opioid analgesic with a more rapid onset and offset of action, which was eliminated independent of hepatic or renal status, and that did not accumulate during long procedures would provide a more tunable and predictable duration of effect in all patient populations. Furthermore, it was projected that ambulatory surgeries were becoming more common, and practitioners needed the effects from anesthesia to clear more rapidly so that patients could be sent home sooner. Thus, an opioid analgesic with the properties of the fentanyl class that could be predictably "turned on and off" nearly instantaneously and was metabolized independent of hepatic or renal mechanisms would be highly differentiated from the agents on the market and support expanding anesthesia needs.

Our discovery program team at Glaxo consisted of biologists, chemists, and scientists skilled at conducting drug metabolism experiments. I co-led the team with Michael James, Ph.D., who also superbly led the pharmacology efforts. Marcus Brackeen, B.A., was the very capable laboratory chemist working alongside me to synthesize novel opioid analgesics. By any standards, our entire program team was small, however, there were several attributes to this program that made it possible to achieve our objective despite our limited resources and experience.

When contemplating the initiation of a new drug discovery program there are several factors that need to be addressed in order to achieve success:

- The vision for the drug needs to be compelling and differentiated from any other drug that is on the market or could make it to market within 10+ yr, such that if success is achieved and the asset gets approved, it will achieve clinical, and hopefully, commercial success.
    - Although this criterion is seemingly obvious, it is unfortunately commonplace for drug discovery programs to commence without a clear and compelling rationale for how the drug discovered from the research program will satisfy an unmet medical need and differentiate from competitor drugs. Within the pharmaceutical industry, this vision for a drug is often referred to as the target product profile.
    - In hindsight, the clinical profile and uses of remifentanil far exceeded our original predictions and expectations. However, the original idea and rationale for an ultrashort-acting opioid, as previously outlined, was sufficiently differentiated from the then-marketed opioid drugs, as well as anything we could detect in competitors' pipelines, and thus served as our impetus to begin this drug discovery program.
- The requirements for efficacy and safety expressed in the target product profile could be met by engaging the proposed biologic target by the drug molecule.
    - We targeted the precedented central μ–opioid receptor, the same receptor engaged by all fentanyl class members, and we were therefore highly confident that biologic target activation would deliver the required efficacy and safety.
- The biologic assays used to test drug candidates are validated and informative.
    - Assays for testing μ–opioid activity *in vitro*, pain tolerance and duration of action *in vivo*, and pharmacokinetic properties both *in vitro* and *in vivo* were all precedented. We could be reasonably assured the data we obtained from these assays would inform our medicinal chemistry efforts. Additionally, based on the data we obtained from the biologic assays, we could be confident that we would know when we successfully identified a molecule that met the desired preclinical profile.
- The chemical starting points need to be robust and tractable.
    - In order to discover a new drug, medicinal chemists need a lead molecule from which to begin synthesis and testing of novel molecules. Ideal lead molecules

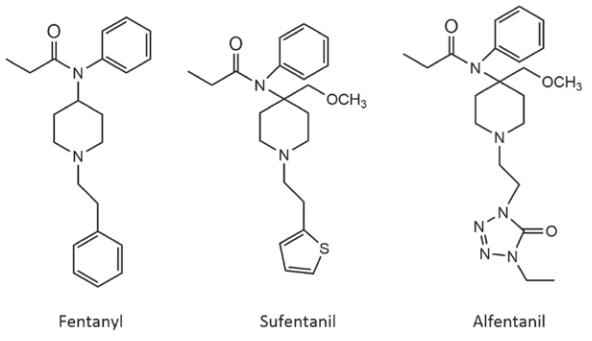

Fig. 1. Chemical structures for fentanyl, sufentanil, and alfentanil. At the commencement of our program in 1987 that led to the discovery of remifentanil these μ-opioid agonists were the contemporary analgesics used as adjuncts to anesthetics for surgical procedures.

have the desired activity for the biologic target (*e.g.*, agonism or antagonism of a receptor), good potency and selectivity for the target, and reasonable drug-like properties for the indication of interest (solubility, lipophilicity, pharmacokinetics, and synthetic feasibility properties). The chemical structure of a lead compound directly influences the biologic target activity, potency, selectivity, and drug-like properties, and medicinal chemists develop structure-activity relationships to understand how changes in structure can affect various properties that must be optimized to achieve the drug's target product profile. In the present case, fentanyl was an outstanding lead from which to pursue our drug candidate. We started from a position of high intrinsic agonist efficacy, excellent target potency and selectivity, good drug-like properties, and there were rich structure-activity relationships based on the fentanyl structure, both in the patent and primary literature, to inform our medicinal chemistry efforts.

In addition to the strong foundation laid down for this program, there was a scientific report that was profoundly useful to me as we started our medicinal chemistry investigations. In 1982, Erhardt *et al.* (American Critical Care, McGaw Park, Illinois) published a paper on the design and synthesis of the ultrashort-acting β-blocker, esmolol.[3] The archetypal β-blocker, propranolol, has a lipophilic naphthalene ring coupled to a hydrophilic side chain that are both essential groups for its high affinity binding to and antagonism of the β-adrenergic receptor. The chemical structure of esmolol has one of the aryl rings of propranolol replaced with a flexible methyl propionate chain (two carbon chain plus a methyl ester). This flexible methyl propionate chain is also hydrophobic and, simplistically, serves to mimic one of the aryl rings of propranolol. One can envision that both the naphthalene ring of propranolol and the aryl methyl propionate groups of esmolol occupy a space within the β-adrenergic receptor that is lined with lipophilic amino acid side chains.

However, the ester group of esmolol is susceptible to enzymatic hydrolysis by esterases which converts the ester into its cognate carboxylic acid metabolite *in vivo*. In essence, the chemical reaction that takes place in the body converts the methyl propionate appendage from a hydrophobic group, which binds well to the β-adrenergic receptor, to a hydrophilic group, which binds poorly to the receptor, and thus turns off the activity of the drug. This concept of introducing a predictable *in vivo* mechanism for metabolism of a drug that obliterates its pharmacologic activity is known in the medicinal chemistry community as the "soft drug" principle (fig. 2). Several marketed drugs spanning different therapeutic areas, including multiple neuromuscular blockers in the anesthesia space, were designed using the "soft drug" principle.[4]

We wanted to use this same concept for the fentanyl class of chemical structures. In order to achieve this goal, we needed to identify a location on the fentanyl-like structures where we could place an ester functional group such that it would bind with high affinity into a lipophilic pocket of the μ-opioid receptor and elicit high potency and intrinsic efficacy (full agonist). In addition, once the ester was metabolized to a carboxylic acid by the action of esterases, the location of the carboxylic acid should render the molecule significantly less able to activate the μ-opioid receptor, thus ablating the efficacy of the drug. Since we knew the ester needed to be a substrate for esterase hydrolysis, it was important that the ester be placed in a sterically unencumbered location on the molecule.

With these considerations in mind, I decided the best position to place an ester was off the piperidine nitrogen of the fentanyl structure, replacing the phenethyl group. We made and tested many molecules that varied the type of ester, as well as the chain length between the piperidine nitrogen and the ester group. Although these structures lacked sufficient *in vitro* potencies at the μ-opioid receptor, approximately 1,000-fold less potent than fentanyl, we discovered that some of these molecules displayed a very short half-life in rats in the analgesic efficacy model. To our satisfaction, the corresponding putative carboxylic acid metabolite of our best molecule in this series was shown to be significantly less potent than the ester in the *in vitro* μ-opioid assay. In other words, we had discovered ultrashort acting μ-opioid

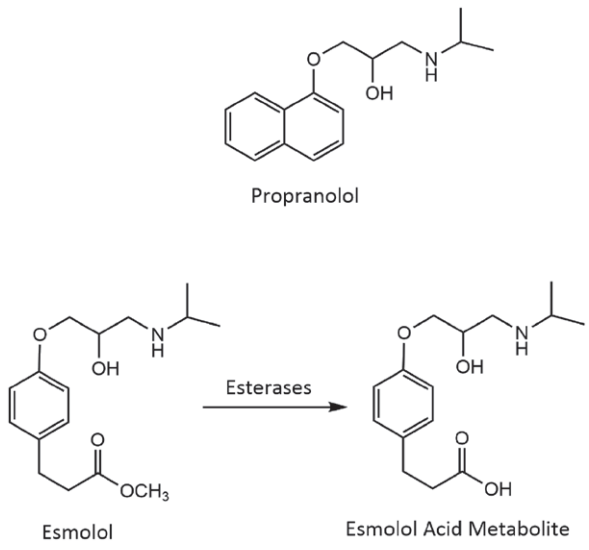

Fig. 2. Chemical structures of propranolol, esmolol, and the carboxylic acid metabolite of esmolol. The discovery of the ultrashort-acting β-blocker esmolol, described in a 1982 publication, was based on replacing the lipophilic naphthalene ring of propranolol with the aryl methyl propionate group in esmolol.[3] Rapid hydrolysis of esmolol's methyl ester by blood esterases produces the esmolol carboxylic acid metabolite which is significantly less active at antagonizing the β-adrenergic receptor. This soft drug medicinal chemistry approach was similarly applied to the discovery of remifentanil.

analgesics that were likely inactivated by conversion of an ester to a carboxylic acid, but we needed to significantly increase the potency of these esters in order to secure a drug candidate suitable for clinical development.

Janssen *et al.* had synthesized a remarkable number of fentanyl analogs in the 1960s and 1970s, and had well defined the structure-activity relationships in this series of analgesics. In their work, they had changed the structures of the piperidine ring of fentanyl and thoroughly explored replacements for the phenethyl chain attached to the piperidine nitrogen. This work prompted me to change the piperidine ring of fentanyl to the carfentanil piperidine structure given the known, significantly enhanced *in vivo* analgesic potency of carfentanil *versus* fentanyl. Carfentanil is one of the most potent piperidine-based analgesics known, and it found use in veterinary medicine to immobilize large game animals. Substituting the phenethyl chain with a methyl propionate chain yielded structure 8, from our original paper, which was remifentanil (fig. 3).

By making the fentanyl to carfentanil piperidine structure change, we were able to increase the *in vitro* μ-opioid potency by approximately 1,000-fold, increase the *in vivo* rat analgesic potency by approximately 700-fold, and maintain the ultrashort duration of action. Importantly, we synthesized the carboxylic acid metabolite of remifentanil and demonstrated it had significantly less *in vitro* potency at the μ-opioid receptor (approximately 500-fold) and was significantly less potent (approximately 350-fold) in the rat analgesic efficacy model *versus* remifentanil. As part of our efforts to further explore the structure-activity relationships of this series of molecules, we synthesized many additional esters. We also evaluated the piperidine ring structures of sufentanil and alfentanil. Through these efforts we were able to discover analgesics with shorter and longer durations of action than remifentanil, as well as identify an analgesic even more potent, both *in vitro* and *in vivo,* than carfentanil. It was a rich, novel, structural vein of μ-opioid agonists.

It was important for us to conduct additional pharmacology and pharmacokinetic experiments with remifentanil—at that time known as GI 87084 within Glaxo—in order to build the confidence required to progress the drug candidate toward clinical development. It was demonstrated that incubation of remifentanil *in vitro* in human whole blood converted it to the carboxylic acid metabolite with a half-life of 37 min. The half-life of the analgesic activity in the rat was much shorter, 15 min, and we attributed this difference to (1) enhanced whole blood esterase activity in rats *versus* humans; (2) tissue esterases that also contributed to remifentanil's conversion to its carboxylic acid metabolite; or (3) a combination of the two. In any event, we confirmed that the primary mechanism for remifentanil's loss of activity was indeed due to hydrolysis of the ester to the carboxylic acid. In a separate, but important, set of experiments conducted in rats, we demonstrated that remifentanil does not accumulate either upon prolonged infusion or upon successive multiple bolus injections. Specifically, when remifentanil was infused into rats for 1 h to achieve maximal analgesic effect, the analgesic effect dissipated upon termination of the infusion with a time course similar to that observed after a single bolus injection. When remifentanil was administered to rats *via* 10 successive bolus injections to achieve maximal analgesic efficacy after each injection, the time to recovery from the analgesic effect remained the same after each injection, which also implied the drug did not accumulate. These sets of experiments further supported the hypothesis that the loss of remifentanil's activity was due almost completely to hydrolysis of the active ester to the significantly less active carboxylic acid metabolite. The pharmacology team at Glaxo further demonstrated that the ultrashort duration of action in rats is similarly ultrashort in dogs. By monitoring the levels of oxyhemoglobin in conscious dogs using pulse oximetry, it was demonstrated that remifentanil suppressed spontaneous respiration, indicative of the known respiratory depressive

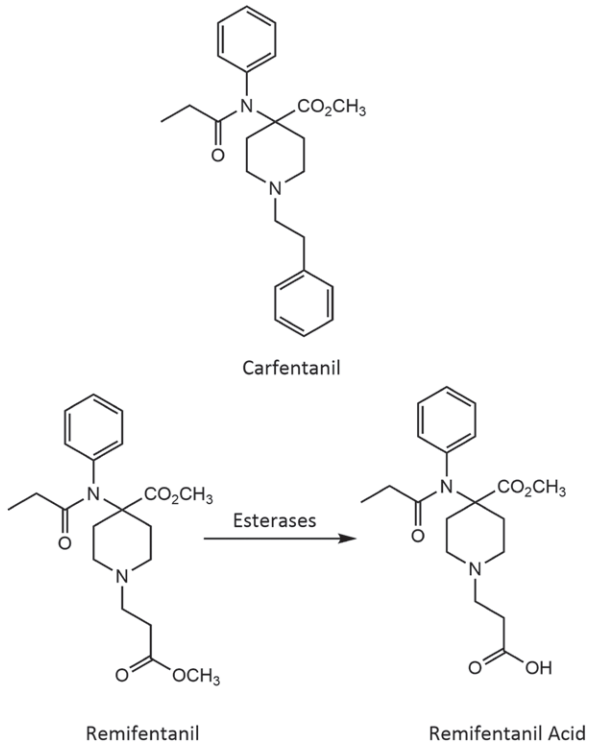

Fig. 3. Chemical structures of carfentanil, remifentanil, and the carboxylic acid metabolite of remifentanil. Replacement of the phenethyl moiety pendant to the piperidine nucleus of carfentanil with a methyl propionate group afforded remifentanil. Hydrolysis of remifentanil's more accessible methyl ester by non-specific, ubiquitous esterases yields the significantly less potent μ-opioid agonist carboxylic acid metabolite. This mechanism for rapid and predictable inactivation of remifentanil's pharmacologic activity enables its use in diverse patient populations and surgical procedures.

effects of μ-opioid agonists, and recovery was very rapid (approximately 15 min) without the need for respiratory assistance. Thus, it was surmised that the known side effects of μ-opioid agonists (respiratory depression, muscle rigidity, and bradycardia) would also rapidly dissipate as remifentanil was converted to its carboxylic acid metabolite. After completing these experiments, the last critical piece of information we needed before progressing the asset into preclinical development (Investigational New Drug application enabling experiments) was to determine whether remifentanil caused histamine release, and it was shown in an *in vitro* assay that it was devoid of that activity.

After our team's work on the discovery and preclinical pharmacologic evaluation of remifentanil, it became the first drug candidate from Glaxo's fledgling United States–based research division to advance into human clinical testing. The first few clinical studies with remifentanil characterized the safety, pharmacokinetics, and preliminary pharmacodynamics. Gratifyingly, remifentanil's human clinical profile largely mirrored what we had seen in the preclinical evaluations in rodents and dogs. Remifentanil displayed dose-dependent analgesic efficacy and class-based side effects with a potency similar to fentanyl, it had a very rapid onset and offset of action (approximately 5 min offset), it did not accumulate during prolonged infusion times, its activities were inactivated by hydrolysis of the ester to the carboxylic acid by nonspecific plasma and tissue esterases, and the dose of remifentanil did not need to be adjusted based on the patient's status (age, gender, body weight, and hepatic or renal status). The characteristics of remifentanil's emerging clinical profile were components that had been designed into the molecule and largely evaluated during the discovery stage and witnessing that profile successfully translate into humans was rewarding.

My involvement with the remifentanil program rapidly diminished as I transitioned to work on a different discovery program and remifentanil was clinically developed by very talented people both within and outside of Glaxo. Remifentanil was approved in the United States in 1996 with initial indications as an analgesic for the induction and maintenance of general anesthesia and for postoperative analgesia in the postanesthesia or intensive care units. Additionally, remifentanil was approved for use during monitored anesthesia care. The expansion of remifentanil's uses for varied clinical procedures during the past 24 yr was something we did not foresee, and I am humbled to witness the stature and importance it has reached within the anesthesia community. Indeed, in a 2013 publication highlighting the most transformative drugs of the past 25 yr, according to surveyed physicians, remifentanil was considered the second most transformative drug, behind propofol, of the 20 drugs classified in the anesthesiology specialty.[5] Attainment of this status was only achieved as a result of the anesthesiology community testing and using remifentanil in innovative ways. Some of the newer uses for remifentanil that are now routine include: conscious sedation procedures such as dental procedures and burn dressing changes, intubations without a muscle relaxant,

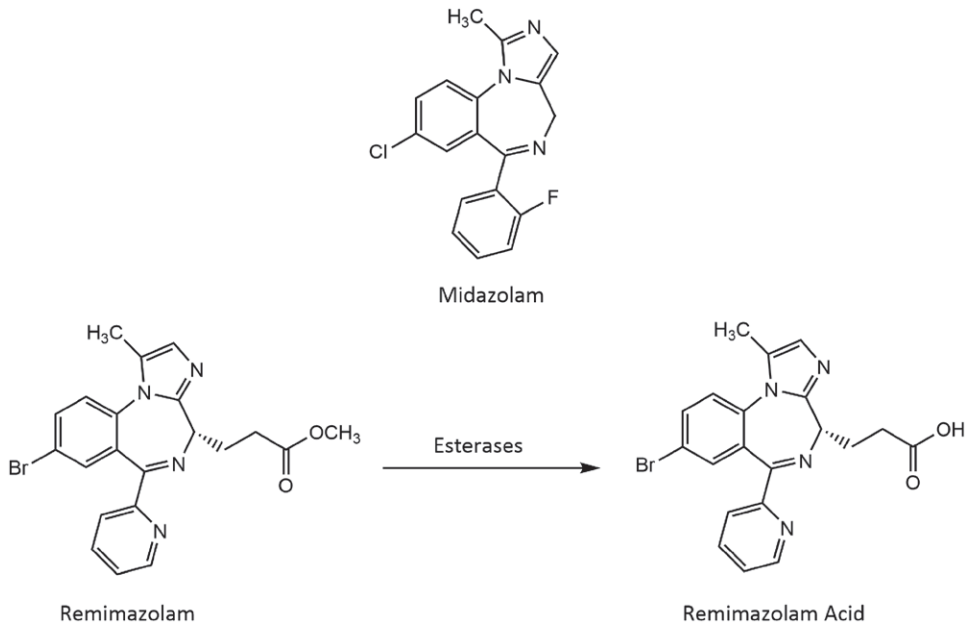

Fig. 4. Chemical structures of midazolam, remimazolam, and the carboxylic acid metabolite of remimazolam. Applying the same medicinal chemistry strategy that led to the discovery of remifentanil, our team at GlaxoSmithKline plc discovered the ultrashort-acting benzodiazepine, remimazolam. Remimazolam manifests similar pharmacologic properties as midazolam but with a predictable, ultrashort duration of action.

obstetric procedures, electroconvulsive therapy, and neurosurgeries where a rapid return to consciousness is needed during the surgery.

In the late 1990s after the success with remifentanil, we applied the same medicinal chemistry principles to discover an ultrashort-acting benzodiazepine. We sought to discover a benzodiazepine that, like remifentanil, was metabolized by esterases and demonstrated a predictable, ultrashort duration of action in all patient populations, and could be used as a next generation anxiolytic–sedative.[6,7] The result of this discovery program led to the identification of remimazolam, in which the mechanism of action is activation of the benzodiazepine receptor in a similar fashion as midazolam, diazepam, and other full agonist benzodiazepine drugs.[8] Remimazolam is inactivated *in vivo* by esterase-mediated hydrolysis of its ester to a much less active carboxylic acid metabolite (fig. 4). Remimazolam was discovered by our team at GlaxoSmithKline plc, spearheaded by the very talented and creative chemist Jeffrey Stafford, Ph.D., and the asset was ultimately acquired by Paion AG, a German-based company who has sold the rights to various companies throughout the world. Remimazolam is and has been studied in the clinic for use in procedural sedation, general anesthesia, and intensive care sedation. A new drug application is currently under review by the U.S. Food and Drug Administration for procedural sedation (filed by Cosmo Pharmaceuticals NV) and has also been filed for approvals in China and Japan.

The modern era of drug discovery, development, and commercialization requires large numbers of talented scientists, physicians, and other professionals to navigate a complex journey from conception to market. It all starts with a compelling idea, followed by the discovery of an excellent molecule, then a robust development program to assess the drug candidate's potential, as well as limitations, to enable regulatory approvals, followed by commercialization and life-cycle management of the drug's clinical use, adoption, and subsequent clinical innovations. Many people have contributed to remifentanil's journey, and I've named a few of the key research scientists who played an integral role in its discovery. I am incredibly grateful to them, as well as to all of those who worked tirelessly to get remifentanil approved, and to the members of the anesthesiology community who have embraced this drug and enabled important and durable uses for it.

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Competing Interests

Dr. Feldman reports the following interests and relationships, all outside the current work: full-time employee of Intarcia Therapeutics, Inc. (Research Triangle Park, North Carolina); board membership at the Chordoma Foundation (Durham, North Carolina); consulting fees from AbbVie Inc. (North Chicago, Illinois); multiple patents; and stock/stock options in Intarcia Therapeutics (Boston, Massachusetts) and GlaxoSmithKline (Brentford, United Kingdom). He was a full-time employee of Glaxo, Inc. when he conducted the work described in the article.

Correspondence

Address correspondence to Dr. Feldman: 6 Davis Drive, P.O. Box 12878, Durham, North Carolina 27709. paul.feldman@intarcia.com. Information on purchasing reprints may be found at www.anesthesiology.org or on the masthead page at the beginning of this issue. ANESTHESIOLOGY's articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

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