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Original Article

NP213 (Novexatin®): A unique therapy candidate for onychomycosis with a differentiated safety and efficacy profile

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Abstract

NP213 (Novexatin®) is a novel antifungal peptide specifically designed for the topical treatment of onychomycosis. NP213 was designed using host defense peptides (HDP), essential components of the innate immune response to infection, as a template. NP213 is a water-soluble cyclic fungicidal peptide that effectively penetrates human nail. NP213 demonstrated a promising preclinical and clinical safety profile, with no evidence of systemic exposure following topical application to the skin and nails. NP213 was efficacious in two phase IIa human trials with 43.3% of patients having no fungi detectable by culture of fragments from NP213-treated nails after 180 days in the first study and likewise 56.5% of patients were culture negative for dermatophytes after 360 days in the second phase IIa study. In both trials, NP213 was applied daily for only 28 days in marked contrast to other topical onychomycosis treatments that require application for up to 52 weeks. Patient reported outcomes from the phase IIa studies were positive with participants recording an improved appearance of their nails after only 14 days of application. All fungi identified in these studies were *Trichophyton* spp. NP213 (Novexatin®) is a promising, highly differentiated peptide-based candidate for the topical treatment of onychomycosis, addressing the infectious cause and cosmetic issues of this very common condition.

Key words: onychomycosis, antimicrobial peptide, clinical trial, tinea, dermatophyte, antifungal.

Introduction

To circumvent the limitations and challenges of drug delivery to the nail, we have taken a biological approach to combatting onychomycosis and designed a novel antifungal peptide, Novexatin® (NP213), specifically for the topical treatment of onychomycosis. NP213 is a synthetic, water-soluble, cyclic antimicrobial peptide that effectively penetrates human nail.[1] NP213 was designed using host defense peptides (HDP) as a template. HDP are essential components of the innate immune response to infection[2,3] and are expressed and produced in skin and nail.[4–7] NP213 is rapidly fungicidal in a water-based topical formulation and demonstrated superior activity to existing antifungal agents under *in vitro* under conditions representative of those in human nail. Significantly, in *ex vivo* human nails, and following only 28 days of daily application, NP213 successfully eradicated dif-

ferent strains of *Trichophyton rubrum* from infected nails, unlike the comparator topical onychomycosis agents ciclopirox and amorolfine. Importantly, there was no evidence of a placebo effect for NP213, as treatment with the water-based vehicle alone did not cause a significant reduction in the number of *T. rubrum* colony-forming units recovered at the end of the experiments. Additionally, NP213 remained bioactive within human nail for at least 11 months following cessation of application.[1] NP213 effectively penetrates human nail in an water-based film-forming vehicle, without the need for penetration enhancers,[8–10] optical brighteners,[11,12] or the use of organic solvents[13–15]; common features in other topical onychomycosis therapeutics and may access the nail by transungual and subungual routes.[16]

Onychomycosis (fungal infection of the nail) is a notoriously difficult to treat infection. Most patients receiving any of the

limited number of currently available treatments (mainly azole- or allylamine-based) often fail to respond or relapse.[17-19] The nail is a highly effective biological barrier; hence, delivery of therapeutic agents to the nail and nail bed is challenging.[20,21] Concomitant tinea pedis (athlete's foot) is common and often a source of re-infection.[22,23] Not surprisingly perhaps, the overall efficacy of current antifungal agents in onychomycosis is poor.[20] Additionally, a number of recent reports have highlighted antifungal resistance in dermatophytes as an emerging problem (including resistance to terbinafine and efinaconazole, itraconazole and cross-resistance).[24-34] Therefore, the need for new, safe, and more effective antifungal agents as onychomycosis therapies is obvious and significant.[35-37] In this paper, we describe the clinical data generated to date for NP213 from phase I and phase IIa clinical studies.

Methods

NP213 (Novexatin) preparation

NP213 was synthesized as an acetate salt (~95% purity) by solid-phase synthesis (PolyPeptide Group, France; Almac Group, UK; Ambiopharm, Inc., USA). NP213 was prepared in amorphous crystalline form as a lyophilized powder and its purity was determined by reversed phase-high performance liquid chromatography. NP213 is a backbone-cyclised homopolymer of 7 L-arginine residues with a net charge of + 7.

Study designs

This paper summarizes our findings from four clinical trials undertaken to assess the safety and efficacy of Novexatin® (NP213). All studies were conducted in accordance with the ethical principles set forth in the Declaration of Helsinki and in compliance with Good Clinical Practice and all applicable regulatory requirements. All subjects were informed of the nature and purpose of clinical studies, and their written informed consent was obtained before study commencement.

Phase I/IIa study

An initial phase I/IIa study (EudraCT No. 2008-001496-29) was a randomised, placebo-controlled, two sequential parts, first-in-human clinical trial with two parts (part one double blind, part two single-blind) to assess safety, tolerability, pharmacokinetics (PK), and pharmacodynamics of NP213 in patients with mild-to-moderate fungal infection of the toenail (25–75% nail involvement). In this study the causative fungus was not specified. Part two (phase IIa) began only after the results of part one (phase I) confirming tolerability and safety were available. Part one enrolled 12 participants with onychomycosis of the toenail that received NP213 or placebo (vehicle) (2:1 ratio), and part two enrolled 48 patients with onychomycosis of the toenail

that received NP213 or placebo (vehicle) (2:1 ratio). A significant number of trial participants (19 out of 42 patients; 45.2%) had more severe onychomycosis than the intention-to-treat population (mild-to-moderate onychomycosis) but were nonetheless included in the study. Study analysis was carried out on all patients including a separate analysis of the intention-to-treat population. A more detailed description of the criteria for all of the trials in this paper can be found in the Supplemental Digital Content.

Second phase IIa study

The second phase IIa clinical trial (ClinicalTrials.gov identifier: NCT02343627) was a randomized, double-blind, placebo-controlled pilot study to assess the safety and efficacy of NP213 solution in patients with mild-to-moderate fungal infection of the toenail (10–50% nail involvement) caused by dermatophytes. The trial enrolled 47 participants that were randomized to receive either NP213 or placebo (3:1 ratio).

Maximum exposure study

A separate stand-alone maximum exposure study was next conducted in order to confirm previous pharmacokinetic data revealing no systemic levels of NP213 following administration to a single target toenail. This study was carried out in addition to the phase I/IIa and second phase IIa studies with an independent patient population. This study intended to ascertain the extent to which NP213 applied to every toe and finger nail daily for 28 days was absorbed systemically. This trial was an open-label, multiple-dose safety and PK trial of 10% (w/v) NP213 solution in a maximal use setting in healthy adult volunteers and patients with severe distal subungual onychomycosis (DSO)[38] caused by dermatophytes of the fingernails and/or toenails (≥50% nail involvement of both great toenails and at least four other toenails). The ideal target product profile of any topical therapy for the treatment of onychomycosis would be to apply the product to all nail and periungual skin as reinfection/recurrence of infection is common[18,19] and can result from subclinical infection of adjacent nails or concomitant tinea pedis (athlete's foot), which is common in patients with onychomycosis.[22,23] This is not possible with current topical onychomycosis treatments. Given the excellent safety profile of NP213 and the lack of systemic absorption of a molecule specifically designed to penetrate nails and not skin, the purpose of this maximal exposure trial was to investigate whether maximal exposure could result in any systemic exposure to NP213 and to determine whether application to all nails and periungual skin could subsequently become part of the treatment regimen.

Fungal identification methods

Infecting fungi were identified using standard methods including microscopy by KOH or Calcofluor white staining and culturing

on selective media as described in Food and Drug Administration (FDA) guidance ((https://www.fda.gov/media/90831/download) and elsewhere.[39-42] A random sample of isolates from study EudraCT No. 2008-001496-29 were subjected to DNA sequencing for more precise identification of the infectious agent using a 314 bp fragment of the fungal large sub-unit ribosomal RNA gene.[43]

Transmission Electron Microscopy (TEM)

Trichophyton rubrum NCPF0118 was prepared for TEM by growing in Roswell Park Memorial Institute (RPMI) 1640 medium for 7 days at 30°C. Fungi were exposed to either NP213 (2000 mg/l) or an equivalent volume of sterile-deionized water for 6 hours at 30°C. For TEM analysis, cells in 2.5% glutaraldehyde solution were dehydrated by passing through ethanol and acetone series before being embedded in wax resin, stained with uranyl acetate/lead citrate stains to improve contrast, sectioned at 90 nm, and mounted onto copper grids. Micrographs were acquired using a JEM-1400 TEM (Jeol USA Inc., Peabody, MA, USA) at the Microscopy and Histology Core Facility at the University of Aberdeen.

Detection of NP213 in human plasma

Detection and quantification NP213 in plasma in the PK component of the studies detailed above was conducted by enzyme-linked immunosorbent assay (ELISA), developed, and validated by NovaBiotics and Charles River Laboratories (Tranent, UK, and Quebec, Canada). The lower limit of quantification (LLOQ) of NP213 in human plasma (normal, hemolysed, or lipemic) in the ELISA was 1.0 ng/ml in the second phase IIa study and the maximal exposure study and met FDA requirements for bioassay sensitivity.

Results

Introduction

As well as a promising efficacy profile established during *in vitro* and *ex vivo* testing,[1] NP213 has been proven to be safe and well tolerated in a panel of preclinical toxicological studies as required to facilitate human studies. Safety and efficacy has been confirmed in clinical studies in humans. NP213 was not absorbed through skin with any detectable drug plasma levels following topical application to the skin and nails of up to 2800 mg over a 28-day period of daily application. NP213 has now been tested in four clinical studies, including three randomized controlled trials (ClinicalTrials.gov Identifiers: NCT02343627; NCT02933879 and EudraCT No. 2008-001496-29), and in total 238 trial participants have been exposed to topical doses of NP213 with no tolerability or safety concerns. This paper summarizes the findings of the human phase I and phase IIa safety and efficacy studies of NP213 in onychomycosis.

Clinical safety

Phase I study

In the phase I study (EudraCT No. 2008-001496-29), systemic exposure was determined on plasma samples by ELISA on 8 trial participants with onychomycosis subjected to a single topical exposure to NP213 solution (10% (w/v)) on an infected toenail. In all cases, NP213 was not detected in plasma, indicating no systemic exposure. No adverse events (AE) were observed in any of the trial participants and there was no evidence of irritation at or around the site of application.

Phase IIa study 1

Following on from this phase I safety study, the initial phase IIa clinical study (EudraCT No. 2008-001496-29), in which NP213 solution (10% (w/v)) was applied daily to a single toenail for 28 days, no serious adverse events (SAE) were reported for any of the 48 participants enrolled. NP213 was not detected in plasma, indicating no systemic exposure. In total, seven AE were recorded that were judged to be possibly related to the study drug in six subjects; five subjects with mild erythema of the skin at the treated toenail of short duration and one case of moderate, untreated headache (one patient on two separate days). In the cases of mild erythema, this was almost evenly distributed between patients receiving (NP213 (three cases) or placebo (two cases) (Table S1). Therefore, the cause of the erythema was not a result of exposure to NP213. The NP213 solution and placebo used in this study also contained 20% (w/v) urea. Urea is generally recognized as safe (GRAS), but there are reports that 5% and 20% urea can cause dermal irritation.[44,45] In cases of onychomycosis, the skin adjacent to the infected nail is often damaged or inflamed, sometimes as a result of concomitant tinea pedis, and this may have made trial participants more susceptible to the irritant effect of urea.[46] Therefore, urea was omitted from the NP213 formulation in subsequent trials and a second phase IIa clinical trial using NP213 solution without urea (ClinicalTrials.gov Identifier: NCT02343627) was conducted to confirm that NP213 did not have any associated safety issues. Overall, in this first phase IIa study, administration of NP213 was very well tolerated by all subjects.

Phase IIa study 2

In the second phase IIa clinical study (ClinicalTrials.gov Identifier: NCT02343627), 47 participants with mild-to-moderate fungal infection of the great toenail received topical once-daily doses of NP213 solution (10% (w/v)) to all infected toenails and 0.5 mm of adjacent skin once-daily for 60 days. There were no SAE in this study and NP213 was well tolerated by all subjects. Of the mild or moderate AE, only one (untreated abdominal

pain) was determined to be possibly related to NP213 (Table S2). No PK analyses were performed in this trial.

Maximal exposure study

Earlier preclinical and clinical studies demonstrated no systemic exposure to NP213 following daily topical exposure to single target toenails, so to confirm the lack of systemic exposure anticipated by dosing multiple nails, a maximal exposure study was conducted in which NP213 solution (10% (w/v)) was applied to all finger and toenails as well as 0.5 mm of adjacent skin once daily for 28 days in seven healthy subjects and 21 participants with severe DSO of the fingernails and/or toenails. NP213 was safe and well tolerated by all participants (healthy and severe DSO) with no SAE and no episodes of application site reactions (skin irritation or sensitization) reported. Importantly, PK analysis revealed plasma concentrations of NP213 were below the LLOQ in all samples tested. Thus, trial participants were exposed to ~2800 mg (2.8×10^9 ng) NP213 over the course of 28 days, with no detectable NP213 found in participants plasma samples. Although this was not an objective of this study, clinical trial sites reported that 14 of the patients with severe DSO had evidence of clear nail growth several months following study completion.

Clinical efficacy

Phase IIa study 1

In the first phase IIa study (EudraCT No. 2008-001496-29), analysis of the culture-based diagnosis of onychomycosis and patient-reported clinical improvement in participants with mild-to-moderate onychomycosis (intention-to-treat population), 84.6% of patients reported clinical improvement after 180 days compared to only 20% on placebo, whereas when the number of culture negative samples were also taken into account, 38.4% receiving NP213 reported improvement and had at least one negative culture over the 180 days, whereas this percentage decreased to 10.0% in the patients on placebo (Fig. 1A). When assessing all of the NP213 treated patients in this study demonstrated that 43.3% of participants reported clinical improvement after 180 days, compared to 31.25% on placebo. When the number of culture negative samples was also taken into account, 43.3% receiving NP213 reported clinical improvement and had at least one negative culture over the 180 days, whereas this percentage was 18.75% in the patients on placebo (Fig. 1B). When assessing mild-to-moderate onychomycosis patients that were culture negative after 180 days of the study (Fig. 1A), a marked difference between those on NP213 and those on placebo was observed at the day 180 time-point (152 days post-cessation of treatment) as 38.4% were culture negative after 180 days, whereas 10.0% of those on placebo were culture negative. An example of improved nail appearance following 28 days of treatment with NP213 solution (10% (w/v)) after 180 days
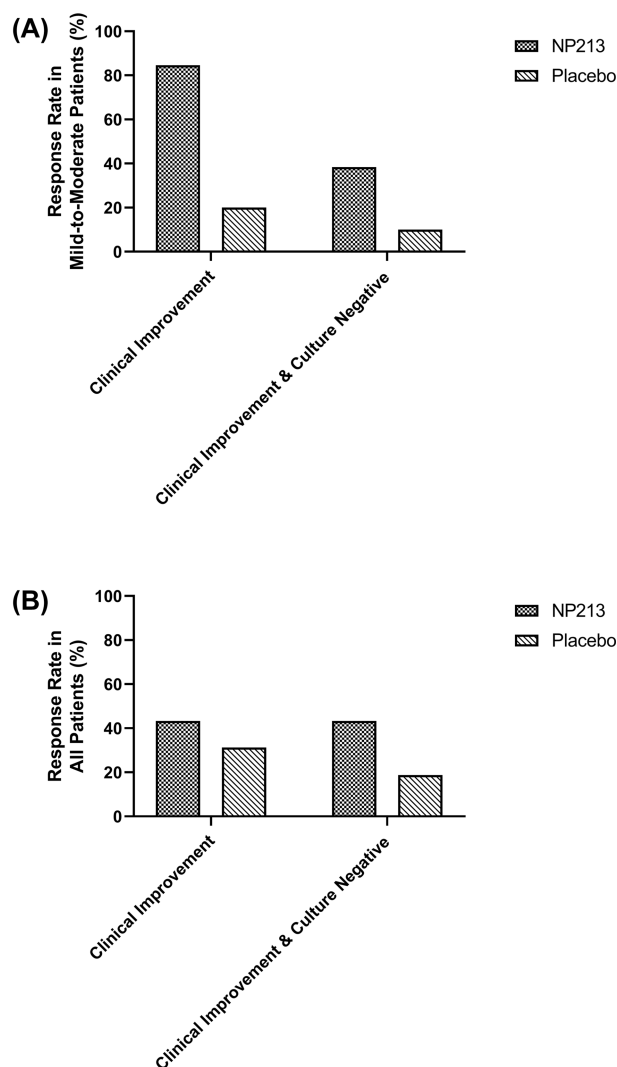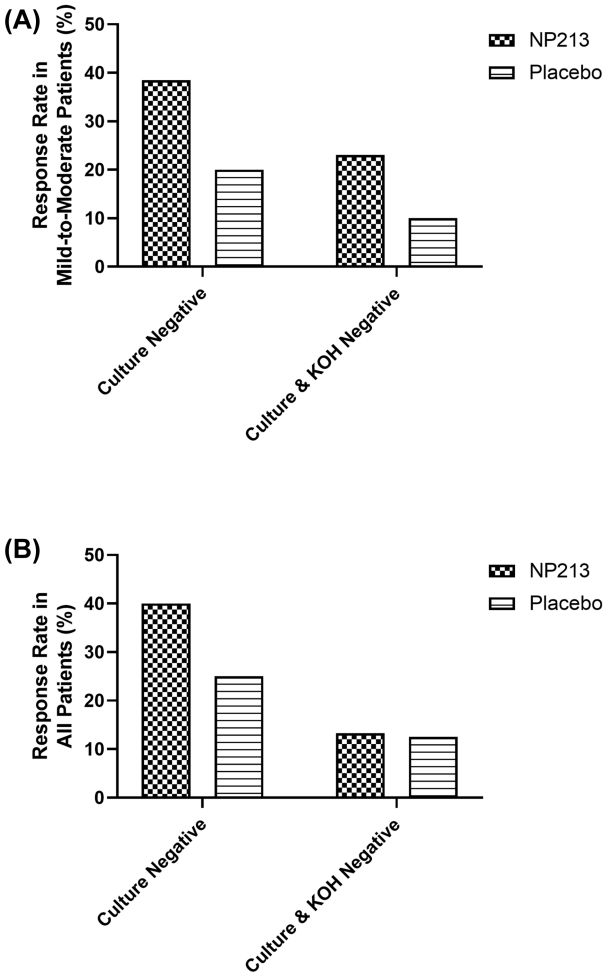

Figure 1. Reported clinical improvement and culture negative after 180 days following daily application of NP213 for the first 28 days to the infected toenail. (A) Mild-to-moderate (intention-to-treat) onychomycosis patients; (B) All onychomycosis patients. At the onset of the study (day 0), all patients (100%) were culture positive and microscopy positive for dermatophytes in the nail material/subungual debris sampled. For culture analysis, samples of nail material and subungual debris were obtained immediately prior to treatment initiation and after 180 d. Treatment with NP213 was conducted on d 1 – 28 of the study. Samples were plated on modified Dermatophyte Test Medium (DTM) agar, Sabouraud Dextrose agar (SDA) and SDA + Chloramphenicol and incubated at 30°C for up to 28 days. Positive cultures were morphologically identified as dermatophytes by an experienced mycologist.

is shown in Fig. 2). This demonstrates that NP213 remained active in the nail for at least 158 day post-cessation of treatment and possibly longer, as an *in vitro* study revealed that NP213 remained active in nails *in vitro* for at least 11 months post-cessation of treatment.[1] Analysis of those patients who were culture negative and microscopy negative (Calcofluor white staining) during the study (Fig. 3) revealed a significant drop in the number of patients who were both culture and microscopy negative; only 23.1% of mild-to-moderate onychomycosis patients and 13.3% of all onychomycosis patients were culture and

Figure 2. Patient nail before treatment for 28 days (A) and after 180 days (B). The nail was treated daily for 28 days with NP213 solution (10% (w/v)). Images were acquired immediately before treatment and 180 post-treatment (152 days following treatment completion).


Figure 3. Reported culture negative or culture and microscopy negative after 180 days following daily application of NP213 for the first 28 d to infected toenails. (A) Mild-to-moderate (intention-to-treat) onychomycosis patients; (B) All onychomycosis patients. At the onset of the study (day 0), all patients (100%) were culture positive and microscopy positive for dermatophytes in the nail material/subungual debris sampled. For culture analysis, samples of nail material and subungual debris were obtained immediately prior to treatment initiation and after 180 days. Treatment with NP213 was conducted on day 1 – 28 of the study. Samples were plated on modified DTM agar, SDA and SDA + Chl and incubated at 30°C for up to 28 days. Positive cultures were morphologically identified as dermatophytes by an experienced mycologist. Microscopy analysis of samples was conducted by fluorescence microscopy following Calcofluor white staining.
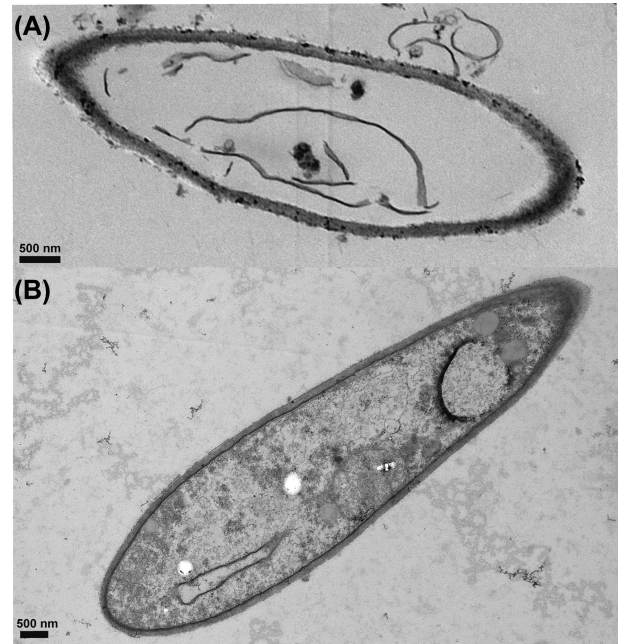
Figure 4. Transmission electron micrographs of *T. rubrum* NCPF0118; A - Exposed to 2000 mg/lNP213 (2 x MIC) at 30°C for 6 h; B – Exposed to an equal volume of sterile-deionized water at 30°C for 6 h. *T. rubrum* NCPF0118 exposed to NP213 (A) was killed resulting in a complete loss of intracellular contents, but with minimal damage to the cell wall. *T. rubrum* NCPF0118 exposed to sdH$_2$O (B) was not killed and an intact cell membrane and normal cell contents are visible.

microscopy negative after 180 days. As this study only lasted for 180 days, we do not believe that microscopy is an appropriate method for determining cure as there is insufficient time for the nail to grow and eliminate fungi, whether dead or alive. It is known from TEM and other studies with NP213 that antifungal activity causes membrane lysis leading to cell death, and this leaves behind intact fungal cell walls (Fig. 4) giving the appearance of 'normal' fungi when analyzed by light or fluorescence microscopy and that these remain in the nail.[1] Therefore, the fungi killed by NP213 would stain with Calcofluor white (as well as KOH or Periodic Acid-Schiff), generating microscopy-positive appearance of fungi within the nail, albeit not viable.

In this study, trial participants reported improvement in the appearance of their nails from day 14 onward, and this was maintained for the remainder of the period of NP213 application. Following cessation of NP213 application, trial participants assessed the appearance of their nails on a weekly basis for a further 9 weeks (Fig. 5). As can be seen from Figure 5, a greater proportion of patients receiving NP213 described an improvement in the appearance of their nails from week 1 until week 9 post-application. In this trial, all microorganisms isolated from trial participant samples by culture were identified morphologically as dermatophytes and by sequencing of a region of the large subunit region of the 28S rDNA gene in a random selection of samples (Table S3) the highest identity was with *T. rubrum* UWFP763.
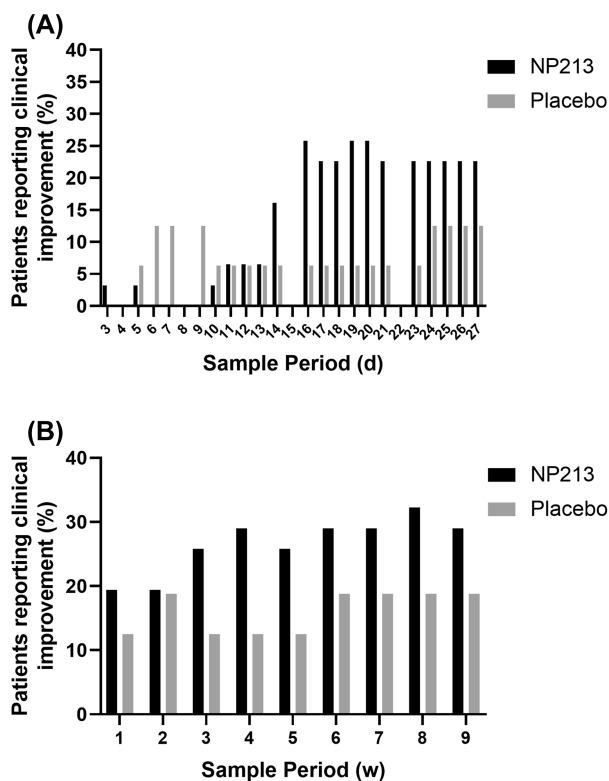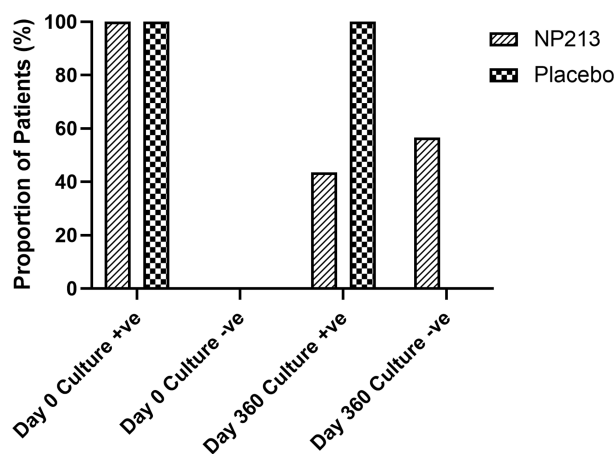
Figure 5. Patient-reported clinical outcomes of nail appearance. Study participants assessed the appearance of their nails during: (A) 28 day treatment phase (daily assessment); (B) Follow-up period after cessation of treatment (weekly assessment), following daily application of NP213 for the first 28 days to infected toenail and follow up for a further 9 weeks after cessation of treatment.

Figure 6. Proportion of patients that were culture negative following daily application of NP213 for the first 28 days to infected toenails in phase IIa Clinical Trial NCT02343627 after 360 days. Samples of nail material and subungual debris were obtained after 360 days and subjected to fungal culture. Treatment with NP213 was conducted on days 1–28 of the study. Positive cultures were morphologically identified as dermatophytes by an experienced mycologist.

Figure 7. Change in the proportion of participants that were culture positive or negative over the 360 days study period following treatment with NP213 for 28 days. Samples of nail material and sub-ungual debris were obtained after 0, 28, 60, 90, 180, 270, and 360 days and subjected to fungal culture. Treatment with NP213 was conducted on days 1 – 28 of the study. Positive cultures were morphologically identified as dermatophytes by an experienced mycologist.
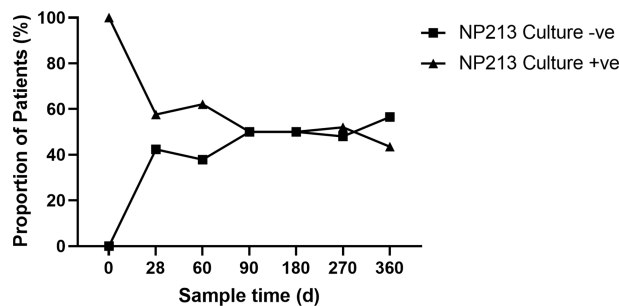
Phase IIa study 2

In the second phase IIa clinical trial (ClinicalTrials.gov identifier: NCT02343627), from which urea was omitted from the formulation, patients were treated with NP213 or placebo for the first 28 days of the study and then followed for a total of 360 days. All fungal specimens from patients were identified morphologically as *T. rubrum*, except one case of *T. tonsurans* and one case of *T. mentagrophytes*. Of the 32 patients that completed the trial until day 360 (23 receiving NP213 and 9 receiving placebo), 56.5% of patients receiving NP213 were culture negative at day 360, whereas none of the patients receiving placebo were culture negative (Fig. 6). When assessing the proportion of patients receiving NP213 over time that were culture negative (Fig. 7), it was demonstrated that even after 28 days (cessation of treatment), 42.4% of patients were culture negative and that this gradually increased over time to a maximum of 56.5% after 360 days.

Discussion

NP213 (Novexatin®) is a unique compound for the treatment of onychomycosis that addresses both the clinical and cosmetic issues associated with onychomycosis (infection eradication and improved appearance of nails) without the need to add additional substances to the formulation such as penetration enhancers, optical brighteners or organic solvents. NP213 is a fungicidal peptide, delivered in a nail- and skin-friendly, water-based formulation that rapidly kills the causative, infecting agents,[1] thereby resolving infection and quickly stopping the ongoing nail discoloration caused by pigments produced by many of the infecting pathogens, for example, *T. rubrum*[47,48] and therefore rapidly improving nail appearance.

In the two phase IIa clinical efficacy studies, NP213 demonstrated favorable cure rates when assessed by culturing infecting fungi from nail samples. However, when cure was also assessed by negative microscopy (Calcofluor white or KOH staining), results were less conclusive. It is our contention that microscopy is not a valid method for determining onychomycosis cure in the case of NP213 for studies that last for ≤ 12

months. This will possibly also be the case for other membrane-active antifungals. NP213 is a membrane-active peptide that lyses the fungal plasma membrane without affecting the cell wall[1] (Fig. 4). Microscopy techniques routinely used for onychomycosis diagnosis (e.g., KOH, Periodic Acid-Schiff, Calcofluor white staining) and in clinical trials for definition of mycological cure simply identify fungal hyphae by staining the fungal cell wall, or nonspecifically within the sample,[42,49,50] and provide no indication of fungal viability.[22,38] Therefore, fungi detected by microscopy may represent fungi that have been successfully killed by treatment with NP213, but that have not been removed from the nail due to insufficient growth of the nail at the time-points used. Microscopy samples from these studies would therefore give positive microscopy results, despite the fungi no longer being viable, severely impacting the apparent efficacy of NP213 when using microscopy as a measure of treatment success. Trial participants will have been confirmed as having onychomycosis by microscopy as well as culture as principal inclusion criteria for entry into the trial, and it is therefore inevitable that fungal hyphae (dead or alive) will be found in nail samples if sufficient time has not elapsed for full nail out-growth, which could take significantly longer than the period of the trial (180 or 360 days in the case of the two phase IIa trials reported herein), especially in the case of involvement of the great toenail; the target nail in most onychomycosis clinical trials. We believe that with culture, considered by ourselves and many others to be the 'Gold Standard' indicator of onychomycosis cure,[38,42,51,52,64,65] there is a much higher degree of certainty that isolated fungi represent identification of an ongoing infection as the fungi are viable. We and others contend that the success or failure of any treatment of onychomycosis is more accurately indicated by using culturing techniques than microscopy.[53] However, we also acknowledge that culture techniques are not perfect,[54-56] as false-negatives can occur (no culturable fungi, but nail remains infected). However, it would be preferable to take multiple samples for culture to reduce the risk of false negatives, rather than rely on microscopy as a definition of cure. Culture is the only one of the current, frequently used methods for onychomycosis diagnosis that determine whether viable fungi are present in the nail, something that is vitally important to know when assessing the efficacy of any fungicidal antifungal agent, including NP213. Current FDA guidance for clinical trials for the treatment of fungally infected nails places considerably less emphasis on microscopy and more on evidence of fungal viability (two negative cultures from the same nail or negative stain with concurrent negative culture) as well as visual appearance of symptom resolution (at least 12 mm or 120 mm^2) increase in clear nail 12 months after first treatment (or complete clearance if < 12 mm distal nail was involved prior to treatment)[57] (https://www.fda.gov/media/90831/download).

Treatment with oral terbinafine or itraconazole remain the Gold Standard treatments for onychomycosis,[38,58] but topical therapies are frequently recommended for the treatment of less

severe cases of onychomycosis.[9,16,53,59] A comparison of the results from the first phase IIa study with other onychomycosis clinical trials to examine mycological cure (negative culture and negative microscopy) demonstrated that NP213 (23.1% mycological cure after 180 days in patients with mild-to-moderate onychomycosis) even though applied for only 4 weeks, compared well with the topical onychomycosis therapies ciclopirox (29 and 36% mycological cure after 48 weeks following daily application for 48 weeks[60]) and amorolfine (8% mycological cure after 10 months following weekly application for 9 months[61]). By assessing cure after 180 days, where treatment with NP213 only took place over the first 28 days of the study, any NP213 on the surface of nails would have been removed, whereas in the case of ciclopirox and amorolfine treatment continued until much closer to the trial end-point and therefore residual ciclopirox or amorolfine could have remained on the surface of the nails (rather than within the nails) and could have adversely affected recovery of remaining infecting fungi from the nails. The rate of mycological cure for NP213 (23.1%) was greater than that achieved for a topical solution of terbinafine (12.7–18.8%) applied daily for either 24 or 48 weeks.[62] A comparison of the levels of cure by culture and mycological cure for topical efinaconazole or tavaborole with NP213 demonstrated that NP213 was not superior, but efinaconazole (53.4 and 55.2% mycological cure) and tavaborole (31.1% mycological cure) were applied for 13 times longer than NP213 (52 weeks compared to 4 weeks).[63,64] If NP213 had been applied for a longer duration, it is likely that superior rates of mycological cure would have been achieved.

If the study participants in the second phase IIa clinical trial had been followed for more than 360 days, it is possible that the proportion of culture negative patients may have increased further from the 56.5% that were culture negative after 360 days, as it is known from in vitro studies that NP213 remains active and bioavailable in the nail for at least 365 days.[1] When trial participants in this study were assessed for negative microscopy (KOH mount), only 13% of patients receiving NP213 were KOH negative after 360 days, further demonstrating that assessment of cure by microscopy is not appropriate for determining the efficacy of NP213 and probably other onychomycosis therapies. Thus, using a definition of cure based on culture alone, a cure rate of 56.5% was achieved for NP213 after 360 days. A comparison with the pivotal terbinafine clinical trial for onychomycosis[65] revealed that after 12 or 24 weeks of oral terbinafine treatment, approximately 80% of trial participants achieved negative culture after 24 weeks (168 days), whereas treatment with NP213 for only 28 days (4 weeks) achieved negative culture in 50% of cases at approximately the same time-point (180 days). Similar to terbinafine, in the two phase III studies with tavaborole, daily topical treatment for 48 weeks achieved negative culture values of 87.0 and 85.4% (57), whereas treatment with NP213 for only 28 days (4 weeks) achieved negative culture values of 56.5% after 360 days. Prolonged treatment with NP213

may have achieved greater rates of negative culture. A phase IIb study to determine the efficacy of NP213 (manuscript in preparation), in which trial participants were exposed to one or two regimens of NP213 for 56 days with daily application, demonstrated that 27% (one regimen) and 36% (two regimens) of patients achieved negative culture at the end of the 365-day study period, which is similar to that achieved in the two phase IIa clinical trials described above.

A range of topical products for the treatment of onychomycosis are available as both prescription-only products (e.g., Jublia® [efinaconazole] and Kerydin® [tavaborole]) or as over-the-counter (OTC) products (e.g., Curanail®, Excilor® and other generic products). Both patients and clinicians often express a preference for topical agents to treat onychomycosis,[64,66-68] but the treatment costs associated with prescription-only products, along with the reduced efficacy compared to oral treatments, is off-putting to many health services and insurers (e.g., Medicare and Medicaid). It was estimated that (using 2016 prices and complete cure as a measure of efficacy) each successfully treated case of onychomycosis cure with tavaborole would cost $176,478.72, whereas each successfully treated case of onychomycosis cure with efinaconazole would cost $72,500.34.[69] Additionally, onychomycosis is more common in the elderly, and they are more often on multiple medications (polypharmacy), which can make the prescription of oral medications for onychomycosis complex, if not impossible.[70,71] However, the cheaper cost of OTC products is generally associated with even lower levels of efficacy, especially those products with unknown active ingredients and those for which no scientific evidence of efficacy is available. There are a number of routes to market for NP213 as an appropriately priced, highly differentiated, safe product with significantly improved efficacy potential over other topical treatments; a solution to a common condition that cannot come soon enough for tens of millions of onychomycosis sufferers.

Supplementary material

Supplementary data are available at *MMYCOL* online.

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