

Double-Blind, Placebo-Controlled Phase I study of PT1.2, a Novel Anti-bacterial Protein (SASP delivery vector)

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12 – 15 September 2010

ABSTRACT

Objective: SASP is a unique antibacterial protein that halts DNA replication and gene expression. SASPject technology delivers SASP genes to target bacteria by modified bacteriophage vectors. SASPject PT1.2 delivers SASP to *Staphylococcus aureus* and is in development for the intranasal decolonisation of *S. aureus*, including MRSA. The primary aim of this study was to assess the safety and tolerability of PT1.2 given intranasally in healthy *S. aureus* carriers and non-carriers.

Methods: This study was a double-blind, placebo-controlled, single to multiple dose escalation Phase I study in a target population of healthy male subjects aged between 18 and 55. The study comprised 2 sequential parts: a single dose phase, Groups 1 (10^7 pfu) and 2 (10^8 pfu) each comprising 16 subjects (8 *S. aureus*/8 non *S. aureus* carriers) randomised in a 3:1 ratio to receive a single dose of PT1.2 or placebo in each nostril; a multiple dose phase, Group 3 (10^8 pfu) comprising 14 *S. aureus* carriers randomised in a 5:2 ratio to receive a single dose of PT1.2 or placebo as 1 application in each nostril twice daily for 5 days and once on Day 6.

Results: There were no serious adverse events (AE) reported during the study and no AE led to discontinuation of PT1.2. Only 1 AE in the single dose and 1 in the multi dose study were considered potentially PT1.2 related. In the single dose study 20 AE were reported by 12 subjects with a lower frequency in the high dose vs the low or placebo dose groups. In the multi dose study 14 AE were reported by 9 subjects. The most common AE was nasopharyngitis. All AE were mild and transient. PT1.2 was not systemically absorbed and though there was a wide range of pre-existing antibody responses to PT1.2 which can be increased with exposure to PT1.2, the increase was variable and the clinical significance appears to be minimal. Nasal tolerability was good.

Conclusions: Intranasal PT1.2 was safe and well tolerated, supporting further clinical development for intranasal decolonisation of *S. aureus*.

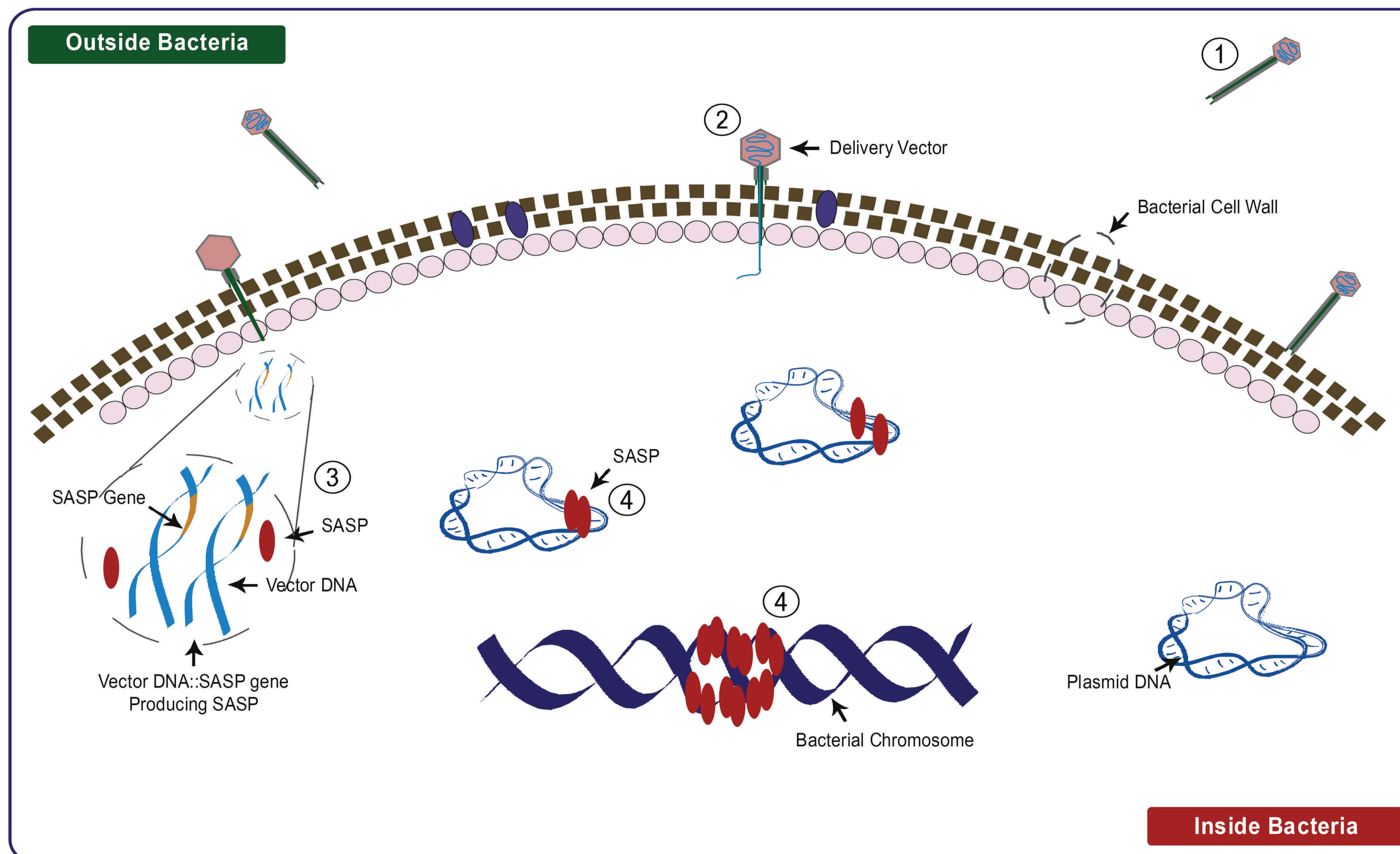
INTRODUCTION

PT1.2 is a component of a unique antibiotic platform technology called SASPject which comprises the delivery of genes encoding one of a group of anti-bacterial proteins into selected bacteria (Figure 1). PT1.2 has been developed as a topical therapeutic for the eradication of *S. aureus*, including methicillin-resistant *S. aureus* (MRSA) for use in hospital infection control.

The active component of the SASPject technology is small acid-soluble proteins (SASP), which are bacterial in origin. These proteins are naturally produced by spore-forming bacteria during the process of sporulation and have a protective role in the dormant spores. In vegetative bacteria SASP act to prevent DNA replication and gene transcription and demonstrate rapid bactericidal activity.

PT1.2 comprises a derivative of the *S. aureus*-specific bacteriophage Φ 11 modified to carry a gene encoding SASP-C from *Bacillus megaterium*. PT1.2 has shown rapid bactericidal activity against >225 *S. aureus*, including MRSA, clinical isolates (up to a 5 log decrease in viable cells within 30 min). An assessment of PT1.2 activity against MRSA and methicillin-sensitive *S. aureus* (MSSA) on *ex vivo* human skin demonstrated a reduction in cell viability to below the limit of detection within 2 h. No bacterial resistance to PT1.2 was demonstrated in serial passaging studies of up to 52 days. In addition, equivalent PT1.2 activity has been demonstrated against both stationary and exponentially growing cells and under widely varying culture conditions. Activity was also unaffected by the presence of mucin, human serum albumin or conventional antibiotics.

Figure 1. Mechanism of action of PT1.2



In vitro studies to assess the possible effects of PT1.2 or SASP on human cells demonstrated no discernable effect by the phage on Human Embryonic Kidney cells, no activity of SASP in eukaryotic yeast cells and no cytotoxic or genotoxic effects of SASP-C against human lymphoblastoid TK6 cells. Toxicokinetic and biodistribution studies performed with rats indicated no systemic exposure to PT1.2 delivered via the intranasal route. In addition, toxicology studies (also in rats) using repeat and single doses of PT1.2 administered via the intravenous and intranasal routes showed that PT1.2 was well tolerated and produced no effects indicative of consistent toxicity at any dose.

This first clinical study was designed to investigate the safety and tolerability of PT1.2 in healthy subjects.

METHODS

This was a Phase I, randomised, double-blind, placebo-controlled sequential single to multiple dose escalation study of PT1.2. The study objectives were to determine the overall safety and tolerability of doses of PT1.2 as a single application (low and high dose) and multiple doses (high dose) when administered in a liquid formulation into each nostril of healthy male volunteers.

The study was carried out in two parts run sequentially: a single dose phase comprising 2 groups of 16 subjects and a multiple dose phase comprising a single group of 14 subjects. A safety data review was conducted between treatment groups, prior to dose escalation.

Single Dose Phase: Sixteen subjects in each of Groups 1 and 2 were

randomised in a 3:1 ratio to receive a single dose of PT1.2 or matching placebo on the morning of Day 1. Each group comprised 8 *S. aureus* carriers and 8 non-*S. aureus* carriers. Group 1 subjects received a single (low) dose of 1×10^7 plaque-forming units (pfu) of PT1.2, or matching placebo, in each nostril and Group 2 subjects received a single (high) dose of 1×10^8 pfu of PT1.2, or placebo, in each nostril.

Multiple Dose Phase: Group 3 comprised 14 subjects (all *S. aureus* carriers) who were randomised in a 5:2 ratio to receive a single (high) dose of 1×10^8 pfu of PT1.2, or placebo, as one application in each nostril twice daily for 5 days, and a further single dose of PT1.2 or placebo in each nostril on the morning of Day 6.

All subjects were followed up for safety for 28 to 35 days.

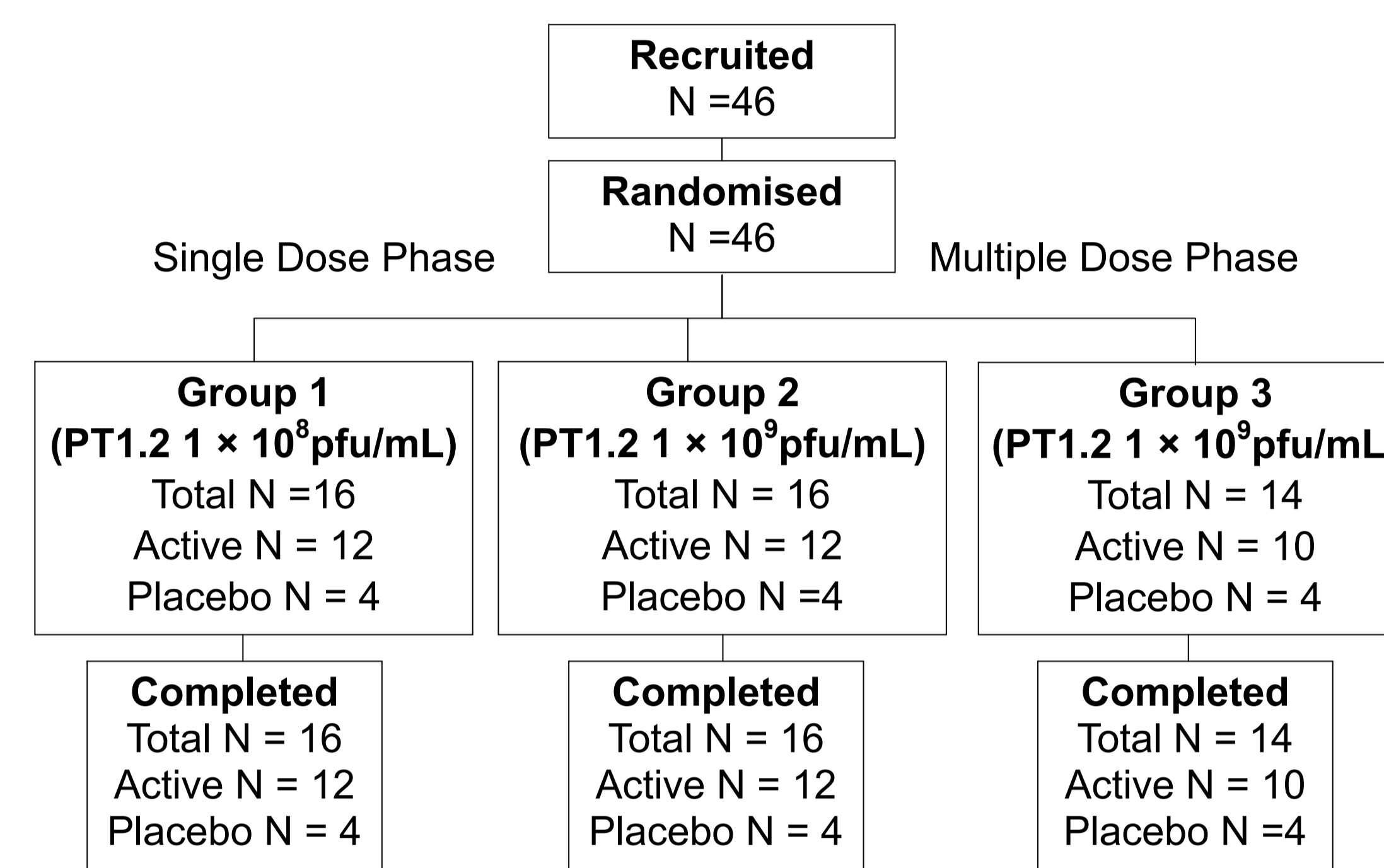
S. aureus nasal carriage status was confirmed by 3 separate nasal swabs taken within a 21 day period prior to dosing.

Blood samples were taken at predetermined intervals throughout the study to monitor subject safety and investigate the PK profile and immunogenicity of PT1.2.

RESULTS

A total of 46 subjects were included in the study. All subjects were healthy male volunteers between 18 and 53 years of age. There were no notable differences in the mean height, weight or BMI between dose groups or between subjects who received active IMP or placebo.

Figure 2. Summary Disposition of Subjects



Treatment-Emergent Adverse Events: There were no serious or severe AEs reported during the study and no reported AE led to discontinuation of IMP for any subject. There was no notable difference between dose groups nor carrier status in the incidence of AEs (Table 1). With the exception of two unrelated events, all events were transient and had resolved by the end of the study. The most common AEs were nasopharyngitis, oropharyngeal pain and headache. No other events were reported by more than one subject.

Table 1. Incidence of Adverse Events

	Single Dose		Multiple Dose	
	Placebo	PT1.2	Placebo	PT1.2
		1×10^7 pfu/nostril	1×10^8 pfu/nostril	1×10^8 pfu/nostril
	(N=8)	(N=12)	(N=4)	(N=10)
No. subjects reporting AEs	5	4	3	7
No. AEs	8	8	4	10
No. mild AEs	8	8	4	10
No. IMP-related AEs	2	0	1	1

Table 2. Nasal Tolerability Scores

	Single Dose		Multiple Dose	
	Placebo	PT1.2	Placebo	PT1.2
		1×10^7 pfu/nostril		1×10^8 pfu/nostril
	(N=8)	(N=12)	(N=4)	(N=10)
0 (normal)	8	11	2	6
1 (hyperaemia)	0	1	1	3
2 (inflammation, mild)	0	0	1	1

Nasal Tolerability: The majority of subjects had a nasal tolerability score of 0 (normal) throughout the study. Table 2, below, indicates the highest scores attributed for each subject. No subjects scored either 3 (inflammation, moderate +/- bleeding) or 4 (inflammation, severe +/- bleeding).

Other safety assessments: For both the single and multiple dose phases there were no notable IMP-related changes in clinical laboratory parameters, vital signs or ECGs for any subject.

PK: Blood samples were taken at various intervals from 10 mins to 24 hours post dosing for PK determination. No PT1.2 was detected at any time interval for either the single or multiple dose groups.

Immunogenicity: The results from this study have demonstrated that there is a wide range of pre-existing antibody responses to PT1.2 or the parental Φ 11 (or potential generic phage component) and that treatment with PT1.2 is capable of boosting this pre-existing immune response. This is to be expected due to the ubiquity of bacteriophages in the environment and in the human body. The study has shown that a proportion of subjects had a response and the absolute level of response was highly variable at all doses.

The significance of the observed increases can not be fully determined without taking the clinical data into consideration; the overall safety of the study showed PT1.2 to be well tolerated and the clinical significance of these immunological responses appeared to have no impact in this study.

CONCLUSIONS

PT1.2 was well tolerated when administered intra-nasally at single dose levels of 1×10^7 pfu per nostril and 1×10^8 pfu per nostril and at a multiple dose level of 1×10^8 pfu per nostril over 6 days (total of 11 doses).

There were no serious or severe AEs reported during the study and no AE led to discontinuation of IMP for any subject. The number of IMP-related AEs was low and there was no dose-related trend in their incidence or frequency. All AEs were considered mild and transient in nature.

In the single dose phase, the *S. aureus* carrier status of subjects had no observable effect on the incidence or frequency of AEs.

There was no notable difference between dose groups in terms of the incidence, frequency or severity of nasal tolerability scores.

There were no notable IMP-related changes in clinical laboratory parameters, vital signs or ECGs for any subject.

Systemic exposure to PT1.2 was non-existent for all subjects following intra-nasal administration.

Overall, these data demonstrate that PT1.2 was safe and well tolerated in the nasal cavity at dose levels of 1×10^7 pfu per nostril and 1×10^8 pfu per nostril.