

TMC278, a new potent NNRTI, with an increased barrier to resistance and favourable pharmacokinetic profile

de Béthune M-P¹, Andries K², Azijn H¹, Guillemont J³, Heeres J², Vingerhoets J¹, Lewi P², Lee E⁴, Timmerman P² and Williams P⁴

¹Tibotec, Mechelen, Belgium; ²Johnson & Johnson Pharmaceutical Research and Development, Beerse, Belgium; ³Johnson & Johnson Pharmaceutical Research and Development, Val de Reuil, France; ⁴Johnson & Johnson Pharmaceutical Research and Development, High Wycombe, UK.

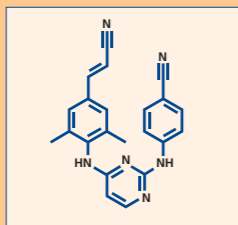
Marie-Pierre de Béthune
Tibotec BVBA
Generaal de Witleaan L11 B3
B2800 Mechelen
Belgium
Tel: +32 15 401 240
mdbethun@tibbe.jnj.com

Introduction

- Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are key components of combination anti-HIV therapy. However, resistance to HIV-1 may rapidly develop during NNRTI therapy, especially if adherence is incomplete.¹⁻³
- There is a need for new NNRTIs that are highly potent against viruses resistant to currently available NNRTIs, and that possess an increased genetic barrier to the development of resistance.

- A new series of NNRTIs, the diarylpyrimidines (DAPYs), has been developed, and members of this series have demonstrated potent activity against NNRTI-resistant virus.^{4,5}

Figure 1. Structure of TMC278



- TMC278 (R278474) is a DAPY compound that is being evaluated as a once-daily oral NNRTI for use in HIV-infected individuals, including those infected with NNRTI-resistant virus.

- The structure of TMC278 is detailed in Figure 1. Its chemical name is (E) 4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylphenyl]amino]-2-pyrimidinyl]amino]benzotrile; the molecular formula is C₂₂H₁₈N₆ and the molecular weight is 366.42 dalton.⁶

- This presentation reports on the *in vitro* antiviral activity of TMC278 and its pharmacokinetics and safety in HIV seronegative human volunteers.

Methods

Cytotoxicity and anti-proliferative activity

- Standard cell-based assays were used to determine the cytotoxicity and anti-proliferative activity of TMC278. MT4 cells were incubated for 5 days with increasing concentrations of TMC278 and cell viability was measured.

In vitro antiviral activity against HIV-1

- A panel of HIV-1 strains harbouring NNRTI resistance-associated mutations was constructed using site-directed mutagenesis and homologous recombination techniques. This panel was used to profile TMC278 and the currently approved NNRTIs, efavirenz (EFV) and nevirapine (NVP).
- In vitro* selection experiments, starting from wild-type (WT) HIV-1/LAI, were performed to study the development of resistance to TMC278. An experimental setting, combining high multiplicity of infection (MOI) and fixed concentrations of the selecting inhibitors, was used to assess virus breakthrough in the presence of TMC278, EFV and NVP. Virus populations selected were further characterized genotypically and phenotypically.
- The antiviral activity of TMC278 in combination with lamivudine, emtricitabine, tenofovir, EFV, indinavir or lopinavir was assessed *in vitro*. Each drug combination was tested at 3 molar ratios using the isobologram method.⁷
- The effect of TMC278 binding to plasma proteins was assessed by determining the median 50% effective concentration (EC₅₀) value of the compound in the presence of 50% human serum in cell culture experiments.

Pharmacokinetics, safety and tolerability

- The pharmacokinetics, safety, tolerability and *ex vivo* antiviral activity of TMC278 were evaluated in 90 HIV seronegative male volunteers in three placebo-controlled, double-blind, randomized trials (6 subjects per dose received TMC278 and 3 subjects per dose received placebo):
CDE-101, single ascending oral dose trial (12.5–50 mg), 27 subjects
CDE-103, single ascending oral dose trial (50–300 mg), 36 subjects
CDE-102, multiple ascending oral dose trial (25–150 mg once daily for 14 days), 27 subjects.

Pharmacokinetic parameters were assessed for up to 6 days after drug intake for CDE-101 and CDE-103, and for up to 9 days after drug intake for CDE-102.

- Plasma concentrations of TMC278 were determined using a validated liquid chromatography – tandem mass spectrometry (LC-MS/MS) method. TMC278 concentrations in urine were determined using a qualified research LC-MS/MS method. *Ex vivo* antiviral activity was measured against the HIV-1/LAI virus.

- Safety and tolerability were recorded through vital signs, ECGs, clinical laboratory tests and adverse event (AE) reporting. Parameters were assessed for 12 days (± 2 days) after drug intake for CDE-101 and CDE-103, and 9 days (± 1 day) after the last drug intake for CDE-102.

Results

Virology

Activity of TMC278 against wild-type HIV-1

- TMC278 showed a median EC₅₀ of 0.5 nM (0.18 ng/ml) against wild-type HIV-1/LAI, and a median 50% cytotoxic concentration (CC₅₀) of 8.1 μM (2.9 μg/ml). This resulted in a selectivity index (ratio CC₅₀/EC₅₀) of 16,000, indicating that TMC278 is a potent and specific HIV-1 inhibitor.
- In the presence of 50% human serum, the fold change in EC₅₀ (FC) was 9, a shift comparable to that observed for EFV.

Activity of TMC278 against site-directed mutant HIV-1 strains with NNRTI resistance-associated mutations

- Among 22 site-directed mutant (SDM) strains with the most prevalent NNRTI mutations, the HIV-1 mutant strain with the lowest sensitivity to TMC278 was the double mutant L100I + K103N, with an EC₅₀ value of 2.7 nM (0.99 ng/ml) and an FC of 5.4 (Table 1).

Activity of TMC278 against recombinant NNRTI-resistant HIV-1 clinical isolates

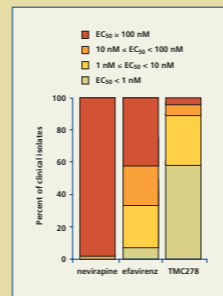
- The antiviral activity of TMC278 was determined against more than 3,500 recombinant HIV-1 clinical isolates with varying phenotypic susceptibility to currently licensed drugs. This includes a large variety of multi-drug resistant variants of which more than 1,500 were NNRTI-resistant i.e. having an FC greater than the biological cut-off for at least one of the current NNRTIs (FC > 8 for NVP or > 6 for EFV).
- 89% of more than 1,500 NNRTI-resistant clinical isolates had an EC₅₀ < 10 nM for TMC278, as compared with 33% and 0% for EFV and NVP, respectively (Figure 2).

Table 1. Activity of TMC278, efavirenz and nevirapine against NNRTI-resistant site-directed mutant HIV-1 strains

NNRTI resistance mutation(s)	NVP ^a		EFV ^a		TMC278	
	Median EC ₅₀ (nM)	Median EC ₅₀ (nM)	Median EC ₅₀ (nM)	FC ^b	Median EC ₅₀ (nM)	FC ^b
Wild-type (WT)	85.0	1.0	0.51	1.0		
L100I	638.0	38.0	0.37	0.7		
K101E	2,467.0	5.6	1.30	2.5		
K103N	5,351.0	39.0	0.35	0.7		
V106A	2,410.0	36.0	0.29	0.6		
Y181C	5,351.0	2.0	1.20	2.4		
Y188L	6,722.0	138	1.6	2.7		
G190A	3,465	11.0	0.30	0.6		
G190S	>10,000	344.0	0.10	0.2		
L100I + K103N	>10,000	> 10,000	2.70	5.4		
K101E + K103N	>10,000	183.0	0.92	1.8		
K103N + Y181C	>10,000	43.0	1.70	3.2		

^a Results for NVP and EFV were generated previously
^b FC is the ratio median EC₅₀ value of the mutant/median EC₅₀ of WT

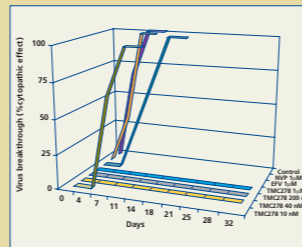
Figure 2. Activity of TMC278, efavirenz and nevirapine against more than 1,500 NNRTI-resistant HIV-1 clinical isolates



In vitro selection of resistant HIV-1 in the presence of TMC278

- In vitro* selection experiments showed that no resistant virus was selected in the presence of ≥ 40 nM TMC278 within 30 days after infection at high MOI (Figure 3). Virus breakthrough was observed in some, but not all, experiments after 10 days in the presence of 10 nM TMC278.
- Viruses selected at 10 nM TMC278 contained up to 8 mutations, including L100I, V106V, Y181Y/C and M230M/I, and showed 7 and 4 FC for EFV and TMC278, respectively.

Figure 3. TMC278 demonstrates an increased genetic barrier to the development of resistance *in vitro*



Activity of TMC278 in combination with other antiretrovirals

- Combination experiments showed no evidence of antagonism between TMC278 and any of the investigated drugs.

Human pharmacokinetics

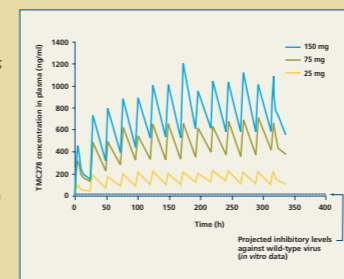
- TMC278 was well absorbed following single oral doses, with dose-proportional increases in exposure up to 200 mg (Table 2).
- At the 300 mg dose the increase in exposure was less than dose proportional.
- t_{max} was achieved at a median of 4 hours post-dose in all dosing groups. Following t_{max}, drug concentrations declined exponentially with time.

Table 2. TMC278 pharmacokinetic parameters in plasma following single oral doses in HIV seronegative volunteers

Dose (mg)	C _{max} (ng/ml)	t _{max} (h)	AUC ₀₋₂₄ (ng•h/ml)	t _{1/2} term (h)
Study TMC278-CDE-101				
12.5	73.1 ± 14.1	4 (4-4)	2,467 ± 526	50.5 ± 21.6
25	149 ± 32.3	4 (2-6)	5,210 ± 2,001	47.7 ± 18.6
50	267 ± 27.4	4 (4-4)	8,872 ± 1,342	44.7 ± 8.7
Study TMC278-CDE-103				
50	226 ± 15	4 (4-4)	6,584 ± 1,881	34.2 ± 12.0
100	482 ± 121	4 (4-6)	15,820 ± 4,568	54.6 ± 17.9
200	807 ± 207	4 (4-6)	28,669 ± 6,876	43.1 ± 13.1
300	944 ± 172	4 (4-4)	32,794 ± 10,352	52.0 ± 17.2

n = 6 subjects per dose; data = mean ± SD except t_{max} = median (range)

Figure 4. Mean plasma concentration profiles of TMC278 following 14 daily oral doses (6 subjects per dose)

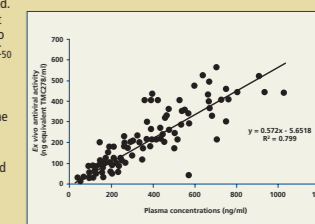


- Drug was eliminated slowly from plasma; the mean terminal t_{1/2} ranged from 34–55h, supporting once-daily administration.
- At 50 mg, the C_{max} averaged 247 ng/ml (674 nM; CV = 12%) and mean AUC₀₋₂₄ was 7,720 ng•h/ml (CV = 25%).
- Following daily oral administration, AUC₀₋₂₄ increased 2.8-fold over 14 days, suggesting an "effective t_{1/2}" of 38h (Figure 4).
- Urinary recovery of unchanged TMC278 was very low (≤ 0.03%). Renal clearance appears to be a very minor route of elimination for TMC278, both after single and multiple dosing.

Ex vivo antiviral activity

- Plasma samples from the volunteers who had received TMC278 during the CDE-102 trial were serially diluted. The antiviral activity of these diluted samples against HIV-1/LAI was determined *in vitro*, and converted to concentration equivalents by comparison with the EC₅₀ of TMC278 against this viral strain.
- Concentrations of TMC278 estimated from this bioassay were highly correlated with the TMC278 concentrations determined by LC-MS/MS assays of the same samples (Figure 5).
- The good correlation suggests that all the antiviral activity of plasma could be attributed to TMC278, and hence that no active metabolites were circulating.

Figure 5. Correlation between *ex vivo* antiviral activity and plasma concentration of TMC278 in the CDE-102 trial



Safety and tolerability

- TMC278 was generally well tolerated after single oral doses up to 300 mg and multiple oral doses up to 150 mg once daily for 14 days.
- There were no serious adverse events (SAEs). Most of the AEs reported during these short regimens were either mild or moderate in intensity. There was no difference in the incidence of AEs in those receiving different doses of TMC278.
- The most commonly reported AEs with TMC278 treatment were nervous system disorders:
 - In single dose trials, headache was reported by 5/42 (12%) subjects receiving TMC278 (all at doses ≤ 50 mg), and by 4/21 (19%) subjects receiving placebo. Dizziness was reported by 5/42 subjects (12%) receiving TMC278 and 2 (22%) of those receiving placebo
 - In the multiple dose trial, headache was reported by 14/18 (78%) subjects receiving TMC278 and by 3/9 (33%) placebo-treated subjects. Dizziness was reported by 5/18 (28%) of those receiving TMC278 and by 2/9 (22%) of those receiving placebo
 - Only headache was considered potentially drug-related as it occurred more frequently after TMC278 than placebo. There were no apparent dose-related trends for any of these AEs.
- No dermatological or abnormal liver function tests were reported.
- There was no indication of any treatment- or dose-related effects on vital signs, ECGs or clinical laboratory tests.

Conclusions

- TMC278 is a potent inhibitor of wild-type and NNRTI-resistant HIV-1 *in vitro*, as demonstrated by the present study.
- TMC278 has an increased genetic barrier to the development of resistance compared to EFV or NVP.
- TMC278 has a favourable pharmacokinetic profile and was generally well tolerated during short-term administration.
- TMC278 warrants further clinical development as a once-daily oral NNRTI.

Acknowledgements

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In recognition of the work of Paul A. J. Janssen, the *Journal of Medicinal Chemistry* will have a special Paul A. J. Janssen memorial issue, March 24 2005.

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