

Supplementary Table S1. Baseline patient and tumor characteristics according to Cmin REGO at C1

| | Cmin REGO C1 <median (n=17) | | Cmin REGO C1≥median (n=17) | | p-value |
|-----------------------------------|---|----------|---|----------|----------------|
| | n | % | n | % | |
| Gender | | | | | 1 |
| Male | 10 | 58.82 | 10 | 58.82 | |
| Female | 7 | 41.18 | 7 | 41.18 | |
| Age, y | | | | | 0.5582 |
| Mean ± SD | 61.52 ± 9.14 | | 58.81 ± 10.44 | | |
| Min-max | 44.74-76.20 | | 36.20-70.80 | | |
| Primary tumor resected | | | | | 0.4729 |
| No | 10 | 58.82 | 12 | 70.59 | |
| Yes | 7 | 41.18 | 5 | 29.41 | |
| Sidedness | | | | | 0.1026 |
| Right | 0 | 0 | 4 | 23.53 | |
| Left | | | | | |
| +Rectosigmoid | 17 | 100.00 | 13 | 76.47 | |
| Grade at initial diagnosis | | | | | 0.7909 |
| 1 | 4 | 26.67 | 6 | 40.00 | |
| 2 | 8 | 53.33 | 7 | 46.67 | |
| 3 | 3 | 20.00 | 2 | 13.33 | |
| Missing | 2 | | 2 | | |
| Stage at initial diagnosis | | | | | 1 |
| I | 0 | 0 | 1 | 5.88 | |
| II | 1 | 5.88 | 0 | 0 | |
| III | 5 | 29.41 | 4 | 23.53 | |
| IV | 11 | 64.71 | 12 | 70.59 | |
| Number of metastatic sites | | | | | 1 |

| | | | | | | |
|---------------------------|--------------|----|-------|----|-------|--------|
| | 1 | 3 | 17.65 | 3 | 17.65 | |
| | ≥2 | 14 | 82.35 | 14 | 82.35 | |
| Metastatic sites | | | | | | |
| | Liver | 16 | 94.12 | 13 | 76.47 | 0.3353 |
| | Lung | 12 | 70.59 | 14 | 82.35 | 0.688 |
| | Lymph nodes | 4 | 23.53 | 8 | 47.06 | 0.2818 |
| | Peritoneal | 3 | 17.65 | 4 | 23.53 | 1 |
| | Bone | 2 | 11.76 | 1 | 5.88 | 1 |
| KRAS | | | | | | |
| | Wild-type | 14 | 82.35 | 11 | 64.71 | 0.4384 |
| | Mutated | 3 | 17.65 | 6 | 35.29 | |
| NRAS | | | | | | |
| | Wild-type | 12 | 85.71 | 12 | 92.31 | 0.8756 |
| | Mutated | 2 | 14.29 | 1 | 7.69 | |
| | Missing | 3 | | 3 | | |
| BRAF | | | | | | |
| | Wild type | 14 | 87.50 | 12 | 85.71 | 1 |
| | Mutated | 2 | 12.50 | 2 | 14.29 | |
| | Missing | 1 | | 3 | | |
| C-reactive protein | | | | | | |
| | Out of range | 10 | 58.82 | 14 | 82.35 | 0.2587 |
| | Normal | 7 | 41.18 | 3 | 17.65 | |
| Albumin | | | | | | |
| | Out of range | 7 | 41.18 | 2 | 11.76 | 0.1175 |
| | Normal | 10 | 58.82 | 15 | 88.24 | |

Supplementary Table S2. Correlation between the M-2 C2 and C1 accumulation ratio and Csum (REGO+M-2+M-5)

| | M-2 C2/C1 ratio | | | | | | | | |
|------------------------------------|------------------------|----------------|-------------|-------------|---------------|-------------|-------------|-------------|-------------|
| | N | Missing | Mean | SD | Median | Q1 | Q3 | Min | Max |
| Csum C1 < 2.5 mg/L | 7 | 2 | 2.04 | 1,62 | 2.25 | 0.67 | 2.35 | 0.44 | 4.46 |
| 2.5 ≥ Csum C1 < 5.5 mg/L | 12 | 0 | 1.33 | 0.79 | 1.38 | 0.60 | 1.91 | 0.28 | 2.51 |
| Csum C1 ≥ 5.5 mg/L | 15 | 6 | 0.66 | 0.33 | 0.70 | 0.31 | 0.76 | 0.23 | 1.17 |

Abbreviations: Csum, Concentration sum of regorafenib, M-2, and M-5 trough concentrations

Supplementary Table 3. RECIST 1.1 assessment according to Csum (REGO+M-2+M-5) ranges at C1 and C2 in the overall TEXCAN population

Patients with clinical progressive disease and/or missing radiological assessment at 4W or 8W has been considered as progressive. Concentrations are in mg/L

A. Best overall response and control rate at week 8 according to C1 Csum

| Best overall response | C1 Csum | | | | | | | |
|-------------------------------------|----------|--------------|-----------|--------------|-----------|--------------|-----------|--------------|
| | < 2.5 | | 2.5-5.5 | | ≥ 5.5 | | Missing | |
| | n | % | N | % | n | % | n | % |
| Stable disease | 5 | 71.43 | 12 | 100.00 | 11 | 73.33 | 14 | 66.67 |
| Progressive disease | 2 | 28.57 | 0 | 0 | 4 | 26.67 | 7 | 33.33 |
| Total | 7 | 12.73 | 12 | 21.82 | 15 | 27.27 | 21 | 38.18 |
| Frequency Missing = 0 P=0.17 | | | | | | | | |

| Control rate at week 8 | C1 Csum | | | | | | | |
|-------------------------------------|----------|--------------|-----------|--------------|-----------|--------------|-----------|--------------|
| | < 2.5 | | 2.5-5.5 | | ≥ 5.5 | | Missing | |
| | n | % | N | % | n | % | n | % |
| Stable disease | 4 | 57.14 | 6 | 50.00 | 5 | 33.33 | 5 | 23.81 |
| Progressive disease | 3 | 42.86 | 6 | 50.00 | 10 | 66.67 | 16 | 76.19 |
| Total | 7 | 12.73 | 12 | 21.82 | 15 | 27.27 | 21 | 38.18 |
| Frequency Missing = 0 P=0.27 | | | | | | | | |

Abbreviations: Csum, Concentration sum of regorafenib, M-2, and M-5 trough concentrations

B. Best overall response and control rate at 8 weeks according to C1 Csum and subsequent Csum assessment at C2

In the TEXCAN clinical trial patients had a CT scan at 4W and 8W. Best overall response corresponds to best CT scan result between 4W and 8W.

| C1 Csum | Best overall response | Subsequent C2 Csum | 8W radiologic assessment |
|-----------|-----------------------|--------------------|--------------------------|
| <2.5 mg/L | SD=71.43% (5/7) | <2.5 mg/L | SD=50% (1/2) |
| | | ≥2.5 and <5.5 mg/L | SD=100% (3/3) |

| | | | |
|------------------------------|----------------------|------------------------------|-----------------|
| | | ≥5.5 mg/L | NA |
| | | Unknown | SD=0% (0/2) |
| ≥2.5 and <5.5 mg/L | SD=100% (12/12) | <2.5 mg/L | SD=50% (1/2) |
| | | ≥2.5 and <5.5 mg/L | SD=66.66% (2/3) |
| | | ≥5.5 mg/L | SD=42.86% (3/7) |
| | | Unknown | NA |
| ≥5.5 mg/L | SD=73.33% (11/15) | <2.5 mg/L | SD=0% (0/1) |
| | | ≥2.5 and <5.5 mg/L | SD=66.67% (2/3) |
| | | ≥5.5 mg/L | SD=60% (3/5) |
| | | Unknown | SD=0% (0/6) |
| Unknown | SD=66.67% (14/21) | <2.5 mg/L | NA |
| | | ≥2.5 and <5.5 mg/L | SD=100% (1/1) |
| | | ≥5.5 mg/L | NA |
| | | Unknown | SD=20% (4/20) |

Supplementary Table 4. Toxicities according to Csum (REGO+M-2+M-5)

Concentrations are in mg/L

| At least one grade ≥ 3 adverse event | Csum C1 | | | | | | | | p=0.5540 |
|---|---------|-------|---------|-------|------------|-------|---------|-------|----------|
| | < 2.5 | | 2.5-5.5 | | ≥ 5.5 | | Missing | | |
| | n | % | n | % | n | % | n | % | |
| No | 0 | 0 | 3 | 25 | 6 | 42.86 | 6 | 28.57 | |
| Yes | 7 | 100 | 9 | 75 | 8 | 57.14 | 15 | 71.43 | |
| Total | 7 | 12.96 | 12 | 22.22 | 14 | 25.93 | 21 | 38.89 | |

Frequency Missing = 1

| SAE at C1 or C2 | Csum C1 | | | | | | | | p=0.1157 |
|-----------------|---------|-------|---------|--------|------------|-------|---------|-------|----------|
| | < 2.5 | | 2.5-5.5 | | ≥ 5.5 | | Missing | | |
| | n | % | n | % | n | % | n | % | |
| No | 4 | 57.14 | 12 | 100.00 | 12 | 80.00 | 16 | 76.19 | |
| Yes | 3 | 42.86 | 0 | 0 | 3 | 20.00 | 5 | 23.81 | |
| Total | 7 | 12.73 | 12 | 21.82 | 15 | 27.27 | 21 | 38.18 | |

Abbreviations: Csum, Concentration sum of regorafenib, M-2, and M-5 trough concentrations

Supplementary Table 5. Dose modifications and treatment interruptions according to Csum (REGO+M-2+M-5) at C1 and C2

Concentrations are in mg/L

| Dose modifications | Csum C1 | | | | | | | |
|--------------------|---------|-------|---------|-------|-------|----|---------|------|
| | < 2.5 | | 2.5-5.5 | | ≥ 5.5 | | Missing | |
| | n | % | n | % | n | % | n | % |
| No | 4 | 57.14 | 10 | 83.33 | 12 | 80 | 13 | 61.9 |
| Yes | 3 | 42.86 | 2 | 16.67 | 3 | 20 | 8 | 38.1 |

p=0.4482

| Treatment interruptions or discontinuations | Csum C1 | | | | | | | |
|---|---------|-------|---------|-------|--------|-------|---------|------|
| | < 2.5 | | 2.5-5.5 | | > =5.5 | | Missing | |
| | n | % | n | % | n | % | n | % |
| No | 3 | 42.86 | 11 | 91.67 | 12 | 80.00 | 8 | 38.1 |
| Yes | 4 | 57.14 | 1 | 8.33 | 3 | 20.00 | 13 | 61.9 |

p=0.0043

| Csum C1 | Cumulative dose of REGO at C1 | | | | | | | |
|-------------------|-------------------------------|---------|---------|--------|------|------|------|------|
| | N | Mean | SD | Median | Q1 | Q3 | Min | Max |
| Csum < 2.5 mg/L | 7 | 2542.86 | 804.27 | 2400 | 1800 | 3360 | 1560 | 3360 |
| Csum 2.5-5.5 mg/L | 12 | 3123.33 | 386.86 | 3360 | 2880 | 3360 | 2280 | 3360 |
| Csum ≥ 5.5 mg/L | 15 | 3136.00 | 395.34 | 3360 | 3080 | 3360 | 2240 | 3360 |
| PK Missing | 21 | 2354.29 | 1021.79 | 2480 | 1600 | 3360 | 480 | 3360 |

p=0.051

Abbreviations: Csum, Concentration sum of regorafenib, M-2, and M-5 trough concentrations; REGO, regorafenib; PK, pharmacokinetic

| | Csum C2 |
|--|----------|
| | p=0.3995 |

| Dose modifications | <2.5 | | 2.5-5.5 | | ≥5.5 | | missing | |
|--------------------|------|-------|---------|----|------|-------|---------|-------|
| | n | % | n | % | n | % | n | % |
| No | 2 | 33.33 | 9 | 75 | 6 | 54.55 | 7 | 63.64 |
| Yes | 4 | 66.67 | 3 | 25 | 5 | 45.45 | 4 | 36.36 |

| Treatment interruptions or discontinuations | Csum C2 | | | | | | | |
|---|---------|-------|---------|-------|-------|-------|---------|-------|
| | < 2.5 | | 2.5-5.5 | | ≥ 5.5 | | Missing | |
| | n | % | n | % | n | % | n | % |
| No | 4 | 66.67 | 11 | 91.67 | 8 | 72.73 | 6 | 54.55 |
| Yes | 2 | 33.33 | 1 | 8.33 | 3 | 27.27 | 5 | 45.45 |

p=0.2289

| Csum C2 | Cumulative dose of REGO at C2 | | | | | | | |
|--------------|-------------------------------|---------|--------|--------|------|------|------|------|
| | N | Mean | SD | Median | Q1 | Q3 | Min | Max |
| Csum < 2.5 | 5 | 2528.00 | 594.24 | 2520 | 2520 | 2560 | 1680 | 3360 |
| Csum 2.5-5.5 | 12 | 2983.33 | 542.98 | 3300 | 2540 | 3360 | 1680 | 3360 |
| Csum ≥ 5.5 | 11 | 2672.73 | 734.04 | 2520 | 2520 | 3360 | 840 | 3360 |
| PK Missing | 11 | 2341.82 | 889.83 | 2280 | 1440 | 3360 | 1200 | 3360 |

p=0.2673

Abbreviations: Csum, Concentration sum of regorafenib, M-2, and M-5 trough concentrations; REGO, regorafenib; PK, pharmacokinetic

Supplementary table 6. Influence of sex and Body Max Index on regoragenib and metabolite exposure.

Concentrations are in mg/L

6A. Impact of sex

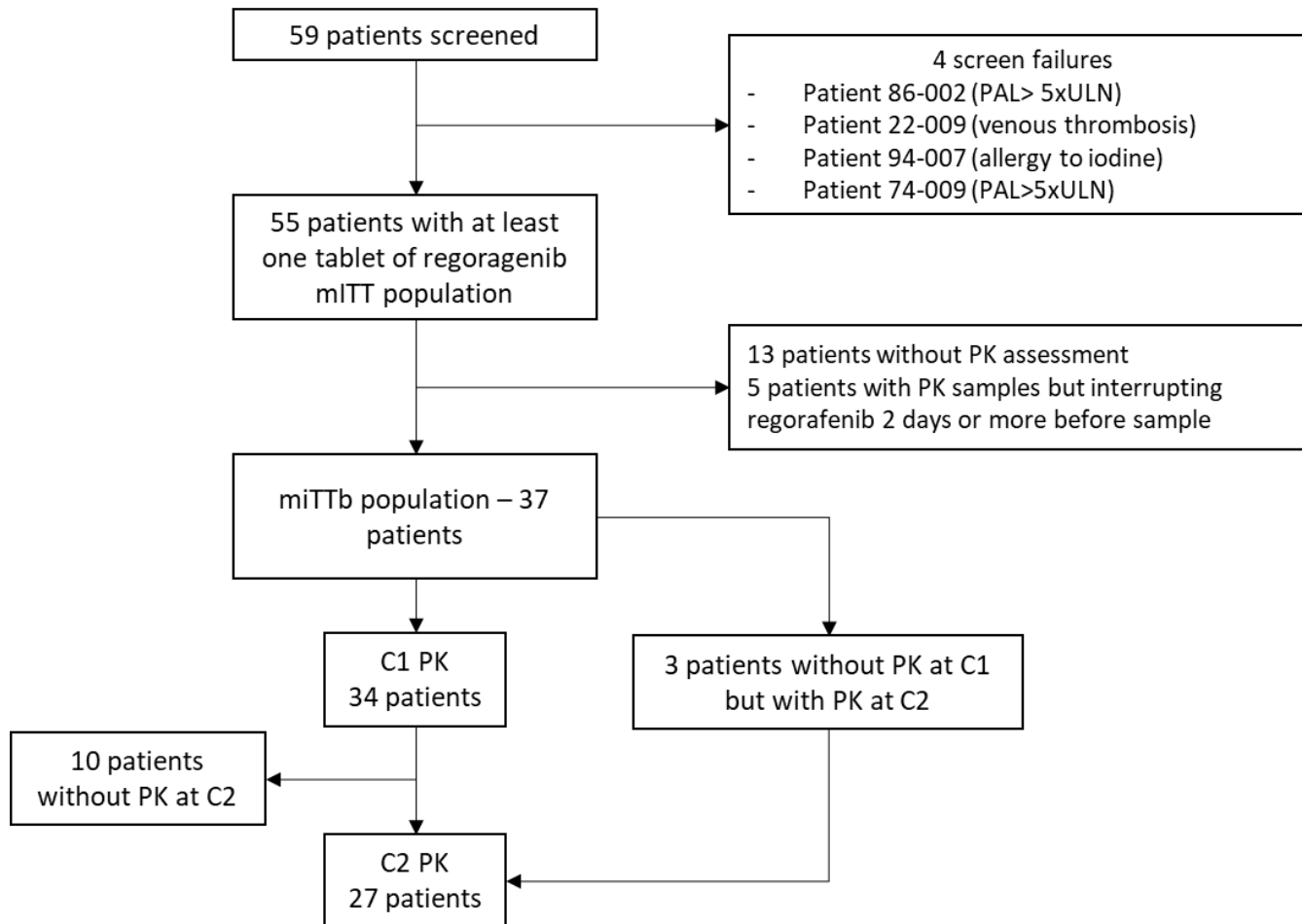
| | cycle1 (n=37) | Male (n=22) | Female (n=15) | pvalue | cycle2 (n=37) | Male (n=22) | Female (n=15) | Pvalue |
|----------------|------------------|----------------|------------------|---------------|------------------|----------------|------------------|--------|
| rego | | | | 0.9442 | | | | 0.9011 |
| N | 34 | 20 | 14 | | 27 | 17 | 10 | |
| missing | 3 | 2 | 1 | | 10 | 5 | 5 | |
| Mean | 2.1 | 2.1 | 2.1 | | 2.1 | 2.02 | 2.25 | |
| Std Dev | 1.17 | 1.13 | 1.27 | | 1.36 | 1.05 | 1.82 | |
| Median | 1.985 | 1.95 | 1.99 | | 1.9 | 1.91 | 1.66 | |
| q1-q3 | 1,03-2,73 | 1,11-2,87 | 1,03-2,6 | | 1,10-2,76 | 1,41-2,33 | 1,07-2,76 | |
| min-max | 0,54-4,84 | 0,54-4,47 | 0,57-4,84 | | 0,43-6,49 | 0,43-3,82 | 0,64-6,49 | |
| regoM2 | | | | 0.2858 | | | | 0.3796 |
| N | 34 | 20 | 14 | | 27 | 17 | 10 | |
| missing | 3 | 2 | 1 | | 10 | 5 | 5 | |
| Mean | 1.69 | 1.54 | 1.9 | | 1.52 | 1.44 | 1.66 | |
| Std Dev | 1.01 | 1 | 1.02 | | 0.91 | 0.98 | 0.8 | |
| Median | 1.436 | 1.34 | 1.97 | | 1.294 | 1.04 | 1.55 | |
| q1-q3 | 0,89-2,49 | 0,77-2,2 | 0,89-2,76 | | 0,77-2,24 | 0,63-2,32 | 1,24-2,16 | |
| min-max | 0,11-3,86 | 0,11-3,56 | 0,56-3,86 | | 0,31-3,34 | 0,31-3,28 | 0,36-3,34 | |
| regoM5 | | | | 0.0209 | | | | 0.1137 |
| N | 34 | 20 | 14 | | 27 | 17 | 10 | |
| missing | 3 | 2 | 1 | | 10 | 5 | 5 | |
| Mean | 1.72 | 1.34 | 2.26 | | 1.59 | 1.29 | 2.09 | |
| Std Dev | 1.19 | 1.06 | 1.2 | | 1.28 | 1.11 | 1.45 | |
| Median | 1.611 | 1.07 | 2.32 | | 1.174 | 0.91 | 1.87 | |
| q1-q3 | 0,79-2,37 | 0,57-1,83 | 1,5-2,85 | | 0,45-2,42 | 0,41-1,8 | 0,95-2,56 | |

| | | | | | | |
|----------------|-----------|----------|-----------|-----------|----------|----------|
| min-max | 0,14-4,65 | 0,14-4,3 | 0,59-4,65 | 0,20-5,10 | 0,2-3,82 | 0,35-5,1 |
|----------------|-----------|----------|-----------|-----------|----------|----------|

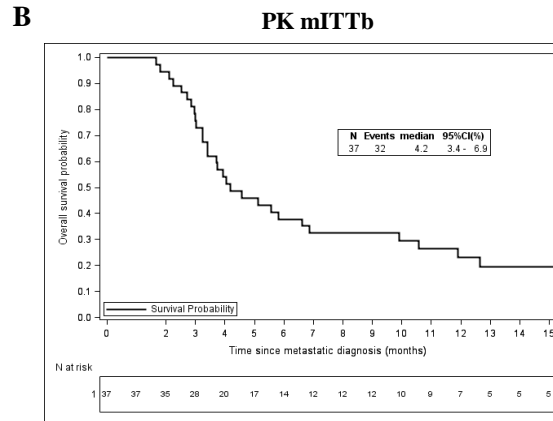
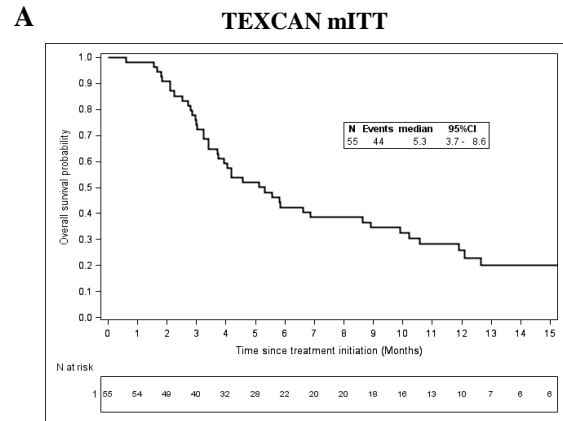
6B. Impact of BMI

| | cycle1 (n=37) | BMI<25 (n=23) | BMI>=25 (n=14) | pvalue* | cycle2 (n=37) | BMI<25 (n=23) | BMI>=25 (n=14) | pvalue* |
|----------------|--------------------------|-----------------------------|------------------------------|----------------|--------------------------|-----------------------------|------------------------------|----------------|
| rego | | | | 0.9025 | | | | 0.029 |
| N | 34 | 20 | 14 | | 27 | 17 | 10 | |
| missing | 3 | 3 | 0 | | 10 | 6 | 4 | |
| Mean | 2.1 | 2.06 | 2.15 | | 2.1 | 1.66 | 2.85 | |
| Std Dev | 1.17 | 1.14 | 1.25 | | 1.36 | 1 | 1.6 | |
| Median | 1.985 | 1.83 | 2.19 | | 1.9 | 1.68 | 2.25 | |
| q1-q3 | 1,03-2,73 | 1,12-2,63 | 1,02-3,01 | | 1,10-2,76 | 0,73-1,91 | 1,96-3,54 | |
| min-max | 0,54-4,84 | 0,57-4,47 | 0,54-4,84 | | 0,43-6,49 | 0,43-3,82 | 1,07-6,49 | |
| regoM2 | | | | 0.3359 | | | | 0.1833 |
| N | 34 | 20 | 14 | | 27 | 17 | 10 | |
| missing | 3 | 3 | 0 | | 10 | 6 | 4 | |
| Mean | 1.69 | 1.84 | 1.47 | | 1.52 | 1.35 | 1.82 | |
| Std Dev | 1.01 | 1.02 | 0.99 | | 0.91 | 0.85 | 0.98 | |
| Median | 1.436 | 1.73 | 1.32 | | 1.294 | 1.24 | 1.97 | |
| q1-q3 | 0,89-2,49 | 0,99-2,57 | 0,41-2,24 | | 0,77-2,24 | 0,77-1,57 | 0,97-2,59 | |
| min-max | 0,11-3,86 | 0,47-3,86 | 0,11-2,99 | | 0,31-3,34 | 0,31-3,28 | 0,49-3,34 | |
| regoM5 | | | | 0.0662 | | | | 0.7441 |
| N | 34 | 20 | 14 | | 27 | 17 | 10 | |
| missing | 3 | 3 | 0 | | 10 | 6 | 4 | |
| Mean | 1.72 | 2 | 1.32 | | 1.59 | 1.52 | 1.7 | |
| Std Dev | 1.19 | 1.18 | 1.13 | | 1.28 | 1.04 | 1.67 | |
| Median | 1.611 | 1.9 | 0.93 | | 1.174 | 1.17 | 1.25 | |
| q1-q3 | 0,79-2,37 | 1,07-2,56 | 0,3-1,89 | | 0,45-2,42 | 0,73-2,29 | 0,34-2,56 | |
| min-max | 0,14-4,65 | 0,46-4,65 | 0,14-3,96 | | 0,20-5,10 | 0,31-3,7 | 0,2-5,1 | |

Supplementary Figure 1. Flow chart of PK TEXCAN study



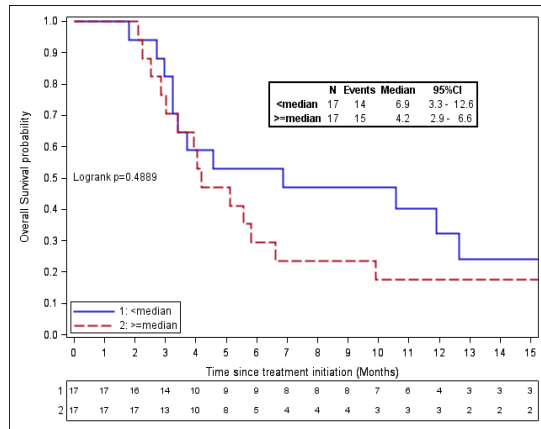
Supplementary Figure 2. OS in the whole TEXCAN mITT population (A) and in the PK mITTb population (B)



Supplementary Figure S3. OS according to M-2 Cmin C1 (A) and M-5 Cmin C1 (B)

A

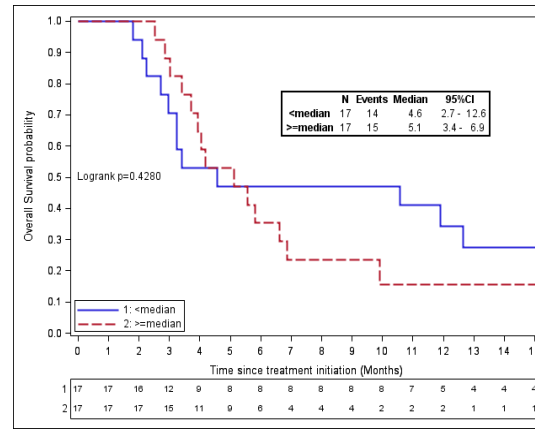
Cmin C1 of M-2 (median=1.44 mg/L)



HR=1.30 (0.62-2.71); p-value=0.4913 (proportional risk hypothesis p=0.44)

B

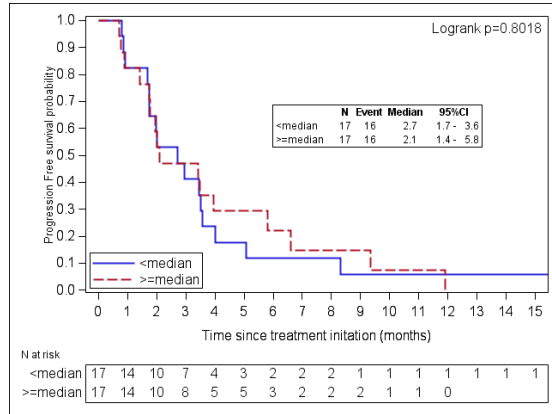
Cmin C1 of M-5 (median=1.61 mg/L)



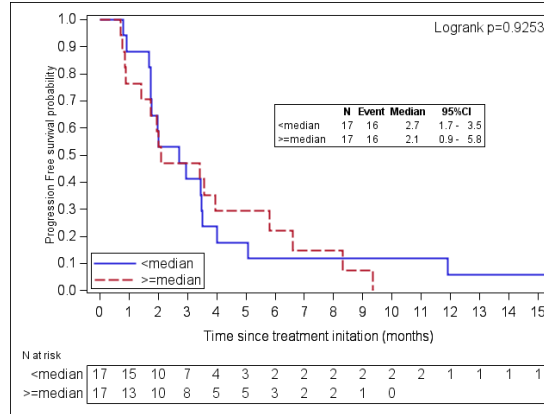
HR=ND (proportional risk hypothesis not valid p=0.025)

Supplementary Figure S4. PFS according to PK parameters in the TEXCAN PK mITTb cohort

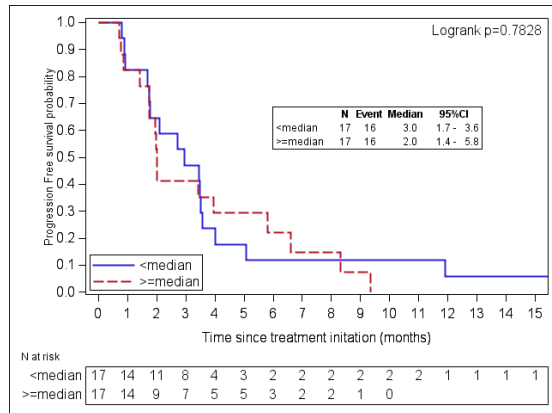
A Cmin C1 of REGO (median=1.99 mg/L)



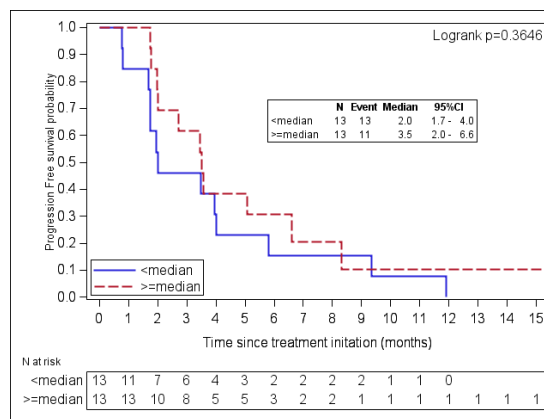
B Cmin C1 of M-2 (median=1.44 mg/L)



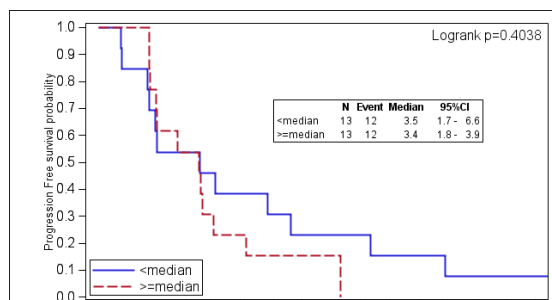
C Cmin C1 of M-5 (median=1.61 mg/L)



D Accumulation ratio C2/C1 of M-2 (median=0.82)



E Accumulation ratio C2/C1 of M-5 (median=0.75)



F Csum C1 (REGO+M-2+M-5) range (mg/L)

