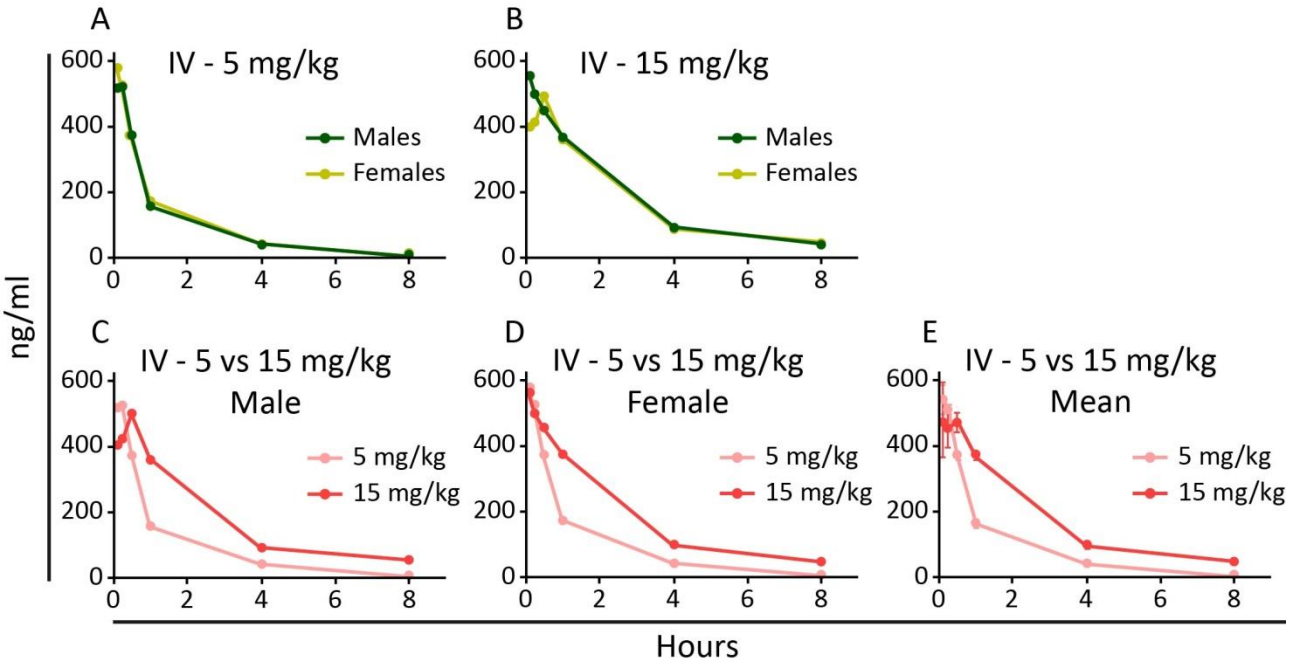

Figure S1. BGA002 pharmacokinetics in mice after single intravenous (IV) administration. Comparison of plasma concentration–time profiles of BGA002 over 8 hours in male and female murine blood, after single IV administration of BGA002 at 5 mg/kg (A) and 15 mg/kg (B). Comparison of plasma concentration–time profiles of BGA002 over 8 hours, after single IV administration of BGA002 at 5 mg/kg and 15 mg/kg in murine blood for males (C), females (D) and the mean of males and females (E). Data for male and female mice at each dose and time point are obtained by the analysis of plasma derived from the blood withdrawn from 3 animals and pooled in the same tube, thus standard deviation values are not available except for the figure E, where the average is calculated on the values of males and females.

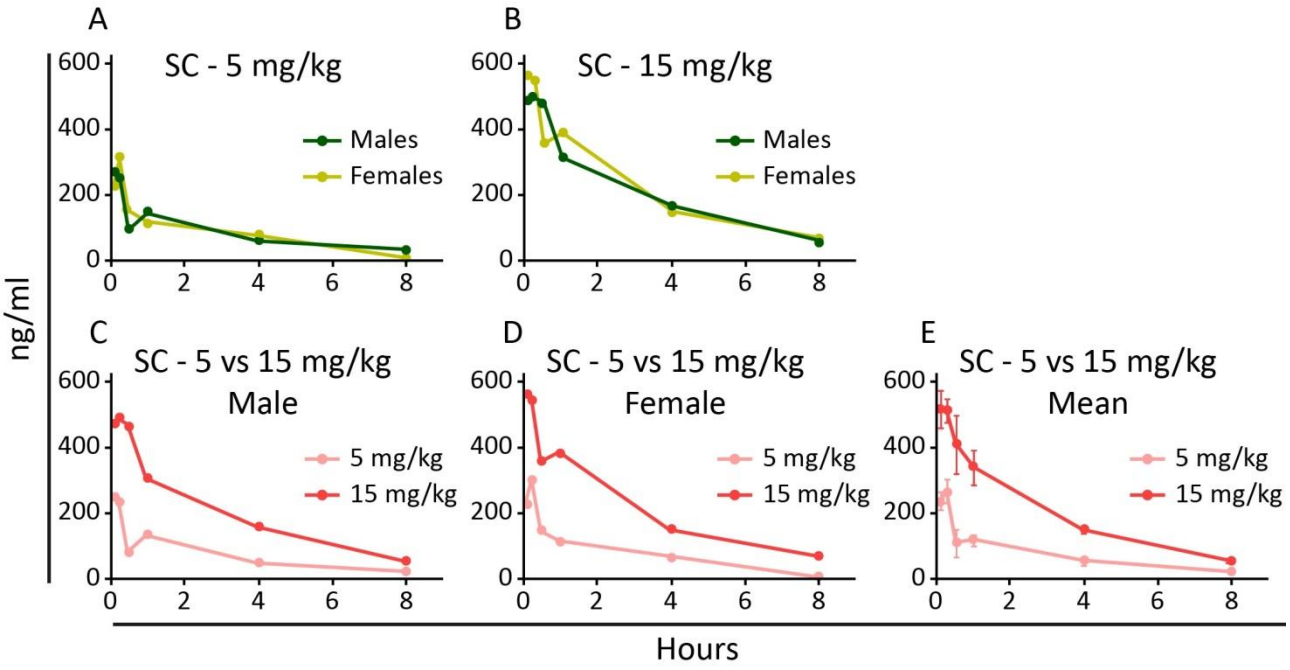

Figure S2. BGA002 pharmacokinetics in mice after single subcutaneous (SC) administration. Comparison of plasma concentration–time profiles of BGA002 over 8 hours in male and female murine blood, after single SC administration of BGA002 at 5 mg/kg (A) and 15 mg/kg (B). Comparison of plasma concentration-time profiles of BGA002 over 8 hours, after single SC administration of BGA002 at 5 mg/kg and 15 mg/kg in murine blood for males (C), females (D) and the mean of values from males and females (E). Data for male and female mice at each dose and time point are obtained by the analysis of plasma derived from the blood withdrawn from 3 animals and pooled in the same tube, thus standard deviation values are not available except for the figure E, where the average is calculated on the values of males and females.

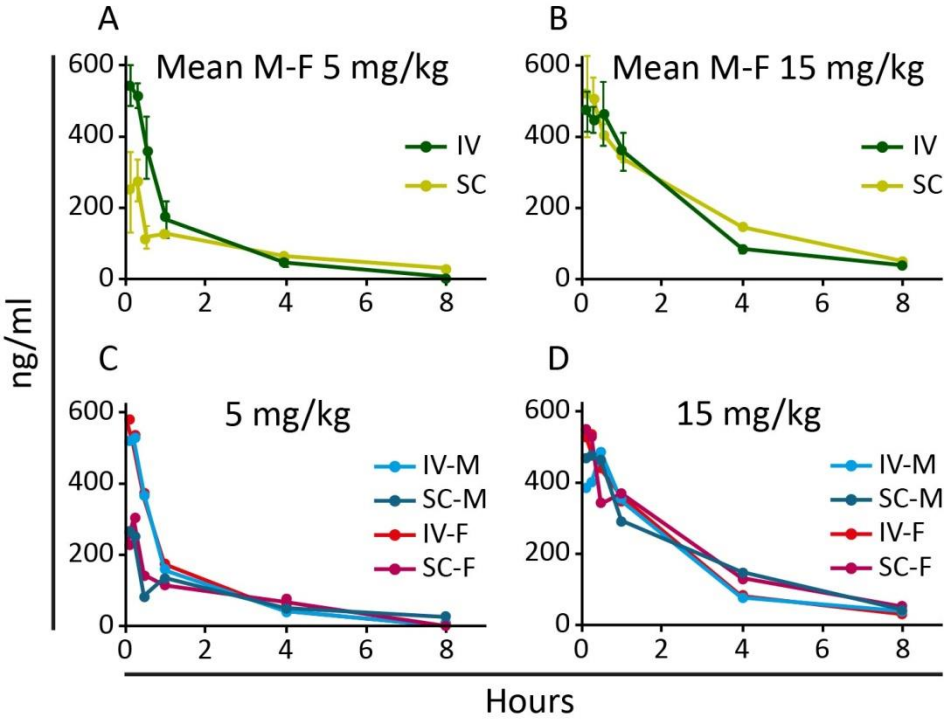

Figure S3. Comparison of BGA002 pharmacokinetics in mice after single subcutaneous (SC) and intravenous (IV) administration, in male and female mice. Comparison of plasma concentration–time profiles of BGA002 over 8 hours after single SC and IV administration of BGA002 at 5 mg/kg (A) 15 mg/kg (B) in male and female murine blood. Comparison of plasma concentration–time profiles of BGA002 over 8 hours after single SC and IV administration of BGA002 at 5 mg/kg (C) and 15 mg/kg (D) reported as mean values of males and females. Data for male and female mice at each dose and time point are obtained by the analysis of plasma derived from the blood withdrawn from 3 animals and pooled in the same tube, thus standard deviation values are not available except for the figure C and D, where the average is calculated on the values of males and females.

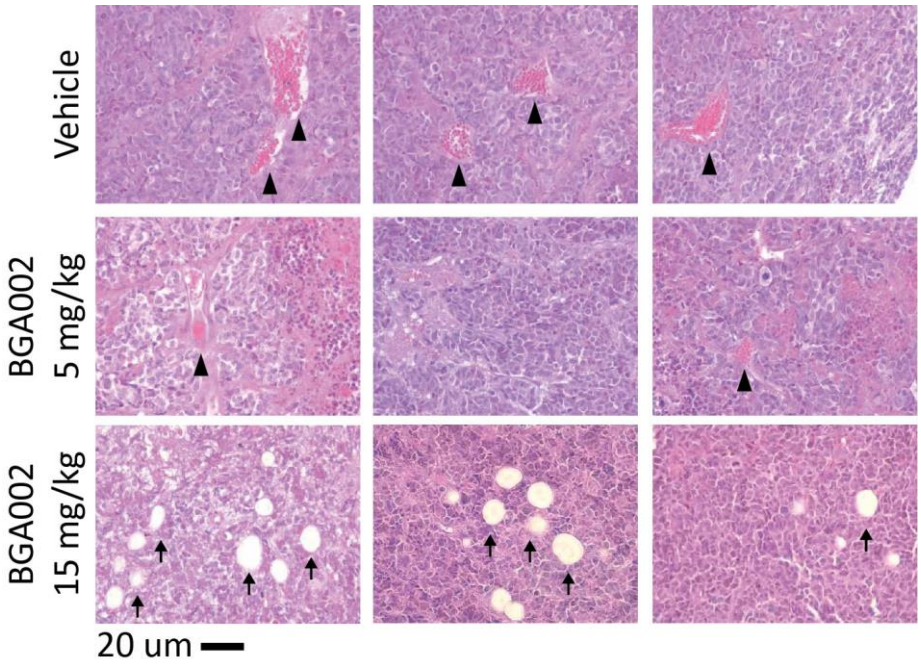

Figure S4. BGA002 inhibits vascularization and cellular density in SCLC tumor masses. Images of tumor masses sections shown are stained with hematoxylin and eosin, after 9 days of once daily systemic administration of BGA002 at 5 or 15 mg/kg/day in a multidrug resistant MNA-SCLC mouse model. Data are reported at the experiment end. Similar results were obtained from three independent mice. Black triangles indicate vascular structures, black arrows indicate areas of low cellular density.

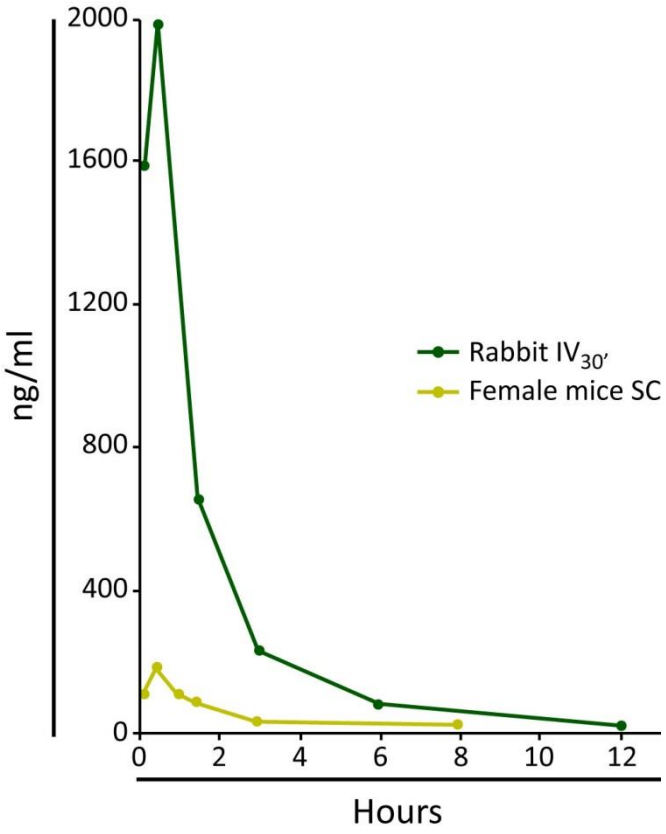

Figure S5. Comparison of plasma concentration in rabbit vs mouse. Comparison of plasma concentration–time profiles of BGA002 at 2.5 mg/kg, after single IV administration in rabbits versus SC administration in mice. For each timepoint the samples from three different animals were pooled in a single tube and analyzed through hybridization-based ELISA, due to technical sample limitation in juvenile animals.

Sex	Dose (mg/kg)	Timepoint (minutes)	Timepoint (hours)	BGA002 concentration in blood (ng/ml)
male	5	5	0,083	509
male	5	15	0,25	520
male	5	30	0,5	370
male	5	60	1	153
male	5	240	4	40
male	5	480	8	<LLOQ
female	5	5	0,083	571
female	5	15	0,25	501
female	5	30	0,5	357
female	5	60	1	170
female	5	240	4	38,2
female	5	480	8	<LLOQ
male	15	5	0,083	397
male	15	15	0,25	411
male	15	30	0,5	492
male	15	60	1	359
male	15	240	4	89,2
male	15	480	8	49,5
female	15	5	0,083	557
female	15	15	0,25	495
female	15	30	0,5	448
female	15	60	1	370
female	15	240	4	95
female	15	480	8	42,5

Table S1 BGA002 concentration in blood after single IV injection in mouse. Raw data of BGA002 blood concentration in mouse is reported for each time point in each sex and all doses tested. Data refers to single IV injection. Lower limit of quantitation (LLOQ).

Sex	Dose (mg/kg)	Timepoint (minutes)	Timepoint (hours)	BGA002 concentration in blood (ng/ml)
male	5	5	0,083	259
male	5	15	0,25	246
male	5	30	0,5	83,1
male	5	60	1	134
male	5	240	4	49,6
male	5	480	8	24,5
female	5	5	0,083	220
female	5	15	0,25	296
female	5	30	0,5	141
female	5	60	1	110
female	5	240	4	65,3
female	5	480	8	<LLOQ
male	15	5	0,083	478
male	15	15	0,25	487
male	15	30	0,5	474
male	15	60	1	306
male	15	240	4	160
male	15	480	8	56,1
female	15	5	0,083	558
female	15	15	0,25	538
female	15	30	0,5	349
female	15	60	1	379
female	15	240	4	144
female	15	480	8	63,3

Table S2 BGA002 concentration in blood after single SC injection in mouse. Raw data of BGA002 blood concentration in mouse is reported for each time point in each sex and all doses tested. Data refers to single SC injection. Lower limit of quantitation (LLOQ).

Dose (mg/kg)	Number of injection	Timepoint (minutes)	Timepoint (hours)	BGA002 concentration in blood (ng/ml)
25	1	10	0,17	731
25	1	30	0,5	1031
25	1	60	1	709
25	1	90	1,5	668
25	1	180	3	454
25	1	480	8	130
7,5	1	10	0,17	332
7,5	1	30	0,5	410
7,5	1	60	1	369
7,5	1	90	1,5	299
7,5	1	180	3	139
7,5	1	480	8	57,1
2,5	1	10	0,17	157
2,5	1	30	0,5	198
2,5	1	60	1	139
2,5	1	90	1,5	110
2,5	1	180	3	34
2,5	1	480	8	26,7
25	28	10	0,17	1284
25	28	30	0,5	576
25	28	60	1	433
25	28	90	1,5	588
25	28	180	3	346
25	28	480	8	125
7,5	28	10	0,17	356
7,5	28	30	0,5	280
7,5	28	60	1	134
7,5	28	90	1,5	227
7,5	28	180	3	171
7,5	28	480	8	61,5
2,5	28	10	0,17	113

2,5	28	30	0,5	182
2,5	28	60	1	117
2,5	28	90	1,5	91,6
2,5	28	180	3	34,6
2,5	28	480	8	25

Table S3 BGA002 concentration in blood after dose escalation in single or repeated SC injections in mouse. Raw data of BGA002 blood concentration in mouse is reported for each time point after single or 28 daily consecutive injections. BGA002 incremental doses range from 2.5 mg/kg to 25 mg/kg.

Dose (mg/kg)	Timepoint (minutes)	Timepoint (hours)	BGA002 concentration in blood (ng/ml)
2,5	10	0,17	1598
2,5	30	0,5	1989
2,5	90	1,5	656
2,5	180	3	231
2,5	360	6	82,2
2,5	720	12	22,6

Table S4 BGA002 concentration in blood after a 30 minutes IV infusion in rabbit. Raw data of BGA002 blood concentration in rabbit is reported for each time point after a 30 minutes IV infusion.

Parameter	Unit	Values
T_{max}	h	0.50
C_{max}	ng/ml	1989
$AUC_{(0-t)}$	h*ng/ml	3499.64
AUC_{inf}	h*ng/ml	3600.09
$t_{1/2\ elim}$	h	3.08
V_d	ml/kg	3086.70
Cl_b	mg/h/kg	694.43

Table S5 Summary of PK parameters after single IV administration in rabbit. Complete PK data are reported after single IV administration of BGA002 in male rabbit treated with BGA002 at 2.5 mg/kg. Each value is reported as mean of multiple single values. Abbreviations: time to reach maximum concentration (T_{max}), highest concentration (C_{max}), area under curve (AUC_{0-t}), AUC from the time of dosing extrapolated to infinity (AUC_{inf}), elimination half-time ($t_{1/2\ elim}$), volume of distribution (Vd), total body clearance (Clb).

SCLC, BGA002 [ug/g]	NB, BGA002 [ug/g]	RMS, BGA002 [ug/g]
27.6	230.0	307.6
165.4	493.7	18.8
6.5	132.8	37.9
11.6	585.9	
61.5		
15.0		
95.5		
130.3		
97.3		
126.8		
267.7		
320,0		
234.9		

Table S6 BGA002 concentration in tumor for SCLC, NB and RMS. Complete data of BGA002 concentration in tumor are reported after SC administration.

SCLC, BGA002 [ug/g]	NB, BGA002 [ug/g]	RMS, BGA002 [ug/g]
341.8	312.0	709.0
390.9	157.4	854.0
295.3	45.6	700.8
260.2	42.9	
51.1	111.5	
107.9	39.8	
177.3	74.1	
228.6		
125.9		
257.2		
598.0		
274.4		
440.5		

Table S7 BGA002 concentration in liver for SCLC, NB and RMS. Complete data of BGA002 concentration in liver are reported after SC administration. P-Value was calculated with Mann-Whitney test.

SUPPLEMENTARY MATERIALS AND METHODS

Animals: rabbits

Pharmacokinetics plasma analysis was performed in juvenile rabbits (New Zealand White rabbit, LAGO: INRA 1077 (Nzw) EOPS) and Wil Research. Aptuit and Farefarma performed respectively ELISA analysis and pharmacokinetics analysis. Animals were maintained according to each CRO internal standard operating procedures.

In-vivo PK/TK experiments in rabbit plasma

The studies were conducted with the support of the following CRO:

- In-vivo phase : WIL Research Europe - Lyon SAS, 329 Impasse du Domaine Rozier, 69210 Saint-Germain-Nuelles, France (now Charles River)
- Bioanalysis: Aptuit Verona Srl, Via Alessandro Fleming, 4 - 37135 Verona, Italy
- Pharmacokinetic Analysis: Farefarma Srl, Via Ferrari, 9 - 28045 Invorio, Italy

The animals used for the studies include Male New Zealand White rabbit (LAGO: INRA 1077 (Nzw) EOPS) (6 weeks old at start of treatment) from Centre LAGO, 01540 Vonnas, France.

The rationale for the dose selection is based on mice studies as described in materials and methods. In particular, for rabbits, which are known to have generally a slower metabolism and a higher exposition when compared to mice, the chosen dose was 2.5 mg/kg. Rabbits were treated with a 30-minute intravenous infusion of 2.5 mg/kg BGA002 (volume administered: 0.625 mL/kg/day and rate of dosing: 1.25 mL/kg/h) using a microflex infusion set introduced into an ear vein (a preliminary compatibility test demonstrated that no compound was retained by the infusion system). Sampling time-points (6 time-points): 10, 30 (before the end of infusion) and 90 minutes, and 3, 6 and 12 hours. Blood sampling time-points were considered from the start of infusion. Blood was sampled individually from the 3 rabbits and plasma was pooled for all 3 animals per time-point.

Control pooled mouse and rabbit plasma (Lithium Heparin-LiHe) are centrifuged for approximately 10 minutes at 3000xg prior to use.