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Photochemical activation of drugs for treatment of therapy-resistant cancers

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Abstract

Resistance to chemotherapy, molecular targeted therapy as well as radiation therapy is a major obstacle for cancer treatment. Cancer resistance may be exerted through multiple different mechanisms which may be orchestrated as observed in multidrug resistance (MDR). Cancer resistance may be intrinsic or acquired and often leaves patients without any treatment options. Strategies for alternative treatment modalities for resistant cancer are therefore highly warranted. Photochemical internalization (PCI) is a technology for cytosolic delivery of macromolecular therapeutics based on the principles of photodynamic therapy (PDT). The present report reviews the current knowledge on PCI of therapy-resistant cancers. In summary, PCI may be able to circumvent several of the major mechanisms associated with resistance towards chemotherapeutics including increased expression of drug efflux pumps, altered intracellular drug distribution and increased ROS scavenging. Current data also suggests PCI of targeted toxins as highly effective in cancers resistant to clinically available targeted therapy such as monoclonal antibodies (mAb) and tyrosine kinase inhibitors (TKI). PCI may therefore in general represent a future treatment option for cancers resistant to other therapies.

1. Background

The ability of cancer to exert resistance towards therapy is one of the main reasons why several tumors remain hard to cure. The resistance may be intrinsic or acquired, and may be the result of several different mechanisms that counteract the action of anticancer treatment. Cancers also often exert cross-resistance towards different therapeutics and/or radiation with distinct mechanisms of action. Cancer resistance towards chemotherapy is long known and is partly the reason why chemotherapy is administered in cocktails of different therapeutics. Resistance has also emerged as a major problem for the novel targeted therapeutics such as tyrosine kinase inhibitors (TKIs) and monoclonal antibodies (mAbs), where acquired resistance is a recognized problem in virtually all treated patients [1]. Treatment regimens are currently being designed to postpone the development of resistance. This is achieved by combining the anticancer therapeutic with a drug which acts on the mechanism of resistance, but also by substituting continuous treatment with fractionated therapy regimens. Even though these approaches clearly have had impact on the time for onset of resistance [2], treatment resistant cancers without any therapeutic options remains one of the major obstacles in cancer therapy, and novel treatment modalities are clearly warranted for resistant cancers.

2. Mechanisms of drug resistance in cancer

The mechanisms of cancer resistance are multiple (Fig. 1) [3, 4]. One of the main resistance mechanisms for drugs with intracellular action points, such as chemotherapeutics and TKIs, is the expression of drug efflux pumps in the plasma membrane. These pumps prevent intracellular accumulation of drugs and subsequent drug action [4, 5]. The adenosine triphosphate binding cassette (ABC) family refers to a group of 48 transport proteins which may function as drug efflux pumps. The ABC transporters may have several drug substrates and they are often the cause of multidrug resistance (MDR). P-glycoprotein (P-gp/MDR1/ABCB1) is a transmembrane transporter widely associated with chemotherapy resistance. Vinca alkaloids, taxanes and antracyclines are all substrates for the P-gp drug efflux pump [3]. Drug inactivation or lack of activation is a second mechanism of which cancer may exert resistance towards cancer therapeutics. This mechanism is highly drug specific and includes inactivation of cisplatin by glutathione [6], but also the silencing of

thymidine phosphorylase which in turn fails to convert the prodrug capecitabine into 5-fluorouracil (5-FU) [7]. Resistance may also be caused by disrupted drug-target interaction due to mutation of the drug target or target pathway. This is an important mechanism of resistance to several targeted therapeutics with both intracellular and extracellular action points. Examples here are imatinib resistance which often is caused by mutation in the BCR-ABL gene disrupting the binding of imatinib to the kinase pocket of the protein [8], and trastuzumab resistance caused by elevated MUC4 expression which masks the trastuzumab-binding epitope of HER2 [9]. Cancer cells may also exert resistance towards targeted therapeutics by activation of alternative non-targeted pathways. The PI3K and MAPK signaling pathways are downstream targets of several therapeutics, and crosstalk between these pathways is well documented to counteract anticancer treatment affecting either pathway. An example is activation of MAPK signaling in rapamycin treatment which counteracts the inactivation of the mTOR-S6K pathway by maintaining growth and surviving signaling. Resistance towards rapamycin has, however, been clinically documented through activation of MAPK signaling which maintains growth and survival-promoting signaling [10]. A fifth main mechanism by which cancer mediates drug resistance is through defects in the control of cell death including insufficiency to undergo apoptosis [11] and increased damage repair or ineffective cell cycle checkpoints [12]. Resistance towards both alkylating agents and topoisomerase inhibitors is *e.g.* shown to be mediated by induction and over expression of the anti-apoptotic protein Bcl-2 [13, 14].

MDR is defined as resistance to multiple drugs with no clear structural similarities and different targets [5]. The ABC transporters, including P-gp, have been highly associated with MDR, however, MDR cells often harbor several mechanisms for drug resistance [15]. The heterogenic composition of cancer is, in addition to the mechanisms outlined above, a major limitation for therapeutic intervention. Cancer frequently displays phenotypic heterogeneity such as the expression of specific receptors on the plasma membrane. High tumor heterogeneity with independent distinct phenotypic populations increases the probability for surviving non-targeted clones following therapeutic treatment which eventually will result in therapy failure [16].

2.1 Strategies to overcome drug-resistant cancer

In theory, drug-resistant cancer may be overcome by two different strategies; circumvention or mechanistic battle towards the cause of the resistance. Circumvention covers the use of drugs, drug combinations or other treatment modalities which are not substrates for the mechanism of resistance. Imatinib resistance caused by mutations in BCR-ABL which disrupts imatinib binding may *e.g* be circumvented by administration of newer BCR-ABL inhibitors that can bind and inhibit the mutant forms of MCR-ABL [17-19]. The search for such drugs or treatment modalities has however in general been of little success due to MDR properties of the resistant cancers together with the high frequency of acquired resistance also towards the circumvention drugs. Several attempts have therefore also been made to pharmaceutically target the mechanism of drug resistance, as in the use of a P-gp inhibitor in combination with the cytostatic drug of interest. The clinical tolerability of such mechanistic battles is, however, still a considerable challenge since such inhibitors have no selectivity towards cancer and therefore inhibit the function of these resistance-related proteins also in normal tissue [5, 20]. We will therefore here propose and describe Photochemical internalization (PCI) as an alternative way of circumventing the mechanisms of cancer resistance.

3. Photochemical internalization

Photochemical internalization (PCI) is a cancer-specific drug delivery system for macromolecular drugs with intracellular action points that are entrapped in endocytic vesicles [21, 22]. PCI is based on amphiphilic photosensitizers such as meso-tetraphenyl chlorine with two sulfonate groups on adjacent phenyl rings ($TPCS_{2a}$) [23], which accumulate in the membranes of endosomes and lysosomes. Light exposure (visible light) induces a photochemical reaction which destabilizes the endo/lysosomal membrane and releases the macromolecular drug of interest into the cytosol where it can reach its target (Fig. 2).

PCI is based on the principles of photodynamic therapy (PDT) where a photosensitizer and light (without a macromolecule) is used for the treatment of several diseases including cancer [24]. Compared to PDT, the main aim of PCI is to induce cancer cell death by the macromolecular drug delivered and not primarily by the photochemical reaction. This has also implications for the photosensitizers used in PCI which are amphiphilic and designed to accumulate in the membranes of

endosomes and lysosomes compared to clinical relevant PDT photosensitizers which in general are more lipophilic [23]. Futhermore, PCI has been shown to induce a deeper tumor necrosis [25, 26] and also a larger vascular effect compared to PDT with the same photosensitizer [26, 27] (Vikdal et al. Manuscript in prep.). These points, together with the increased selectivity which may be provided by the macromolecular drug indicate PCI as a more effective cancer modality compared to PDT. PCI has in addition been indicated to induce an immunogenic reaction which may be important for the treatment response [28, 29]. The anticancer properties of PCI are dependent on sufficient light delivery and several different strategies may be utilized to overcome this possible obstacle. This includes: (1) light exposure through the surface of the skin, most relevant for small and superficial tumors [26, 30, 31], (2) light exposure through different endoscopes, relevant for tumors in close proximity to the pathway of these endoscopes [32, 33] (clinical PCI trial currently enrolling patients ; Clinical Trials.gov Identifier: NCT01900158) and (3) interstitial light exposure, relevant for larger tumors [30, 34] (clinical PCI trial currently enrolling patients; ClinicalTrials.gov Identifier: NCT01606566). PCI has, in addition, been shown highly effective in combination with marginal surgery where the operation wound was subjected to light exposure (tumor bed sterilization) prior to closure [35]. Sufficient light administration should therefore not be recognized as a major limitation for clinical utilization of PCI.

The endo/lysosomal membrane provides a physical barrier for the cytosolic delivery of several targeted macromolecular drugs and many drug delivery systems have been developed to overcome this physical barrier including the use of polyethylenimine (PEI) [36], pH-sensitive liposomes [37], chloroquine [38] or saponin [39] to destabilize the endo/lysosomal membrane. Lack of cancer selectivity is, however, a recognized problem for these delivery systems and this has limited their clinical utilization. In contrast, PCI represents a tumor-selective approach for endosomal release. The cancer-specific properties of PCI may be divided in three; (1) the 2-3 fold enhanced retention of photosensitizer in tumor tissue compared to most normal tissue [23, 40], (2) the site-directed light exposure and (3) the selectivity provided by the macromolecular drug to be delivered [41]. Drugs suitable for PCI-mediated delivery have little toxicities as monotherapies *per se* since they are subjected to substantial lysosomal degradation. Hence, toxicity of these drugs in normal cells will also be prevented due to reduced accumulation of photosensitizer in normal tissues, no light exposure and hence absence of a PCI effect."

PCI has been documented highly effective for the delivery of a variety of macromolecular drugs both *in vitro* and *in vivo*, and PCI of the large size chemotherapeutic drug bleomycin has been shown highly promising in clinical testing (ClinicalTrials.gov Identifiers: NCT00993512 and NCT01606566). Preclinical *in vitro* studies have indicated PCI as a treatment modality for drug-resistant cancers covering model systems for all the main mechanisms of drug resistance and will be described below.

3.1 PCI circumvents resistance provided by drug efflux pumps

Drug efflux pumps including P-gp are major contributors to the development of MDR cells. The substrates for P-gp are diverse, however, they share some properties including molecular size (300-2000 Da), electronegativity and polaryability [42] in addition to high hydrophobicity [43]. PCI introduces the possibility for therapeutic cancer treatment with hydrophilic macromolecular drugs that are not substrates for drug efflux pumps and therefore circumvents MDR mediated through such pumps. Administration of hydrophilic macromolecules with intracellular action points (without PCI) has little clinical potential since these drugs, to a large extent, are degraded in lysosomes before they have exerted their therapeutic effect [44]. The efficacy of PCI in MDR cells has been demonstrated by PCI of the protein toxin gelonin in the MDR cell line MES-SA/Dx5 with acquired resistance to doxorubicin induced by increased expression of P-gp [45]. Even though the macromolecular drugs utilized with PCI are not substrates for drug efflux pumps the photosensitizers utilized may be affected. Several photosensitizers have been indicated as substrates for drug efflux pumps including pheophorbide A [46]; chlorin e_6 [47] and 5-ALA induced PpIX [46-48]. The amphiphilic photosensitizers utilized in PCI such as $TPPS_{2a}$ and $TPCS_{2a}$ are, however, not substrates for the ABC transporters as demonstrated both for ABCG2 [49] and P-gp [45, 50]. PCI generally includes three components; a macromolecular drug, an amphiphilic photosensitizer and light at the appropriate wavelength. None of these components are subjected to cellular efflux through the ABC family of transporters and PCI should therefore represent a treatment strategy for MDR cancers.

3.2 PCI counteracts drug inactivation in doxorubicin-resistant breast cancer

Doxorubicin resistance has generally been associated with P-gp expression, however, altered intracellular distribution of doxorubicin is also a recognized

mechanism of decreased doxorubicin sensitivity [51]. Doxorubicin has been shown to accumulate in acidic endosomes and lysosomes in MDR cells compared to non-resistant cells where the drug is found in the nucleus. This is due to enhanced acidification of lysosomes in some drug-resistant cancer cells, resulting in lysosomal trapping of weak bases, such as doxorubicin [51, 52]. Since doxorubicin exerts its main therapeutic effect in the nucleus, this lysosomal accumulation reduces its cytotoxic effect. Lou et al. therefore postulated that PCI could reverse doxorubicin resistance in MDR cells [53]. Indeed, PCI was shown to induce doxorubicin sensitivity in the MDR cell line MCF-7/ADR to a comparable level as observed in the non-resistant parental MCF-7 cell line. Release of doxorubicin from acidic compartments and subsequent translocation to the nucleus was indicated as the mechanism for resistance reversal (Fig. 3). PCI was therefore suggested as an alternative treatment to overcome resistance of the MDR MCF-7/ADR cells to weak base chemotherapeutics. Chloroquine and omeprazole have also been shown to inhibit endo/lysosomal sequestration and thereby increase the effect of doxorubicin [54]. The tumor specific properties of PCI (site directed light exposure and tumor-localized photosensitizer) should, however, provide a clear clinical advantage compared to other less specific methods for endo/lysosomal escape.

3.3 PCI overcomes resistance to ROS generating therapy

Several anticancer drugs as well as ionizing radiation induce reactive oxygen species (ROS) as a part of their mechanism of action, and the defense mechanisms towards ROS are associated with resistance towards both ionizing radiation and chemotherapy [55-57]. Generation of ROS is also an important part of the mechanisms of PCI since photochemically-induced ROS in the endo/lysosomal membrane is responsible for the cytosolic drug release. PCI of the protein toxin gelonin has, however, been shown to overcome ROS resistance in an *in vitro* model, MES-SA/Dx5 cells, resistant to both radiation therapy and PDT [50]. Increased expression of the ROS-scavenger proteins GPx1 and GPx4 was together with attenuated p38 signaling indicated as the mechanism of ROS resistance in this model. It is not clear why PCI of gelonin seems to be unaffected by the ROS resistance mechanism present in the MES-SA/Dx5 cell line. The distance from ROS production in the endo/lysosomal membrane to GPx1/GPx4, which is reported to localize to the cytosol, mitochondria, nucleus and endoplasmic reticulum (ER), may however contribute to resistance

escape [50]. Thus, although the photochemical effect is attenuated in the MES-SA/Dx5 cells by increased expression of GPx1 and GPx4 the photochemical effect on rupturing endocytic vesicles appears unaffected. This is supported by a report indicating that that much of the photocytotoxicity of $TPPS_{2a}$ may be due to relocalization to ER during light exposure [58].

3.4 PCI of targeted toxins in models resistant to targeted therapy

The therapeutic management of cancer has in the last decade been moving from conventional treatment with chemotherapeutic drugs to more personalized treatment where the patients receive specific drugs based on cancer cells' expression of specific genes or proteins. The current intense focus on cancer genomics aims together with the development of specific molecular inhibitors and antibodies to develop highly personalized cancer treatments based on the specific cancer fingerprint. Lack of cytotoxicity of such cancer specific drugs is, however, a major obstacle and prolonged treatment eventually causes resistance in virtually all patients as observed with the BCR-ABL inhibitor imatinib, HER2-targeted antibody trastuzumab and the EGFR targeted TKI erlotinib [1]. The TKIs and mAbs currently available for cancer treatment inhibit their target-protein signaling pathways as one of their main mechanisms of action. Resistance towards these mechanisms may be overcome by utilizing the targeting drug as a delivery moiety for another cytotoxic compound such as in treatment with targeted toxins or targeted nanoparticles loaded with drugs. As long as the receptor is still internalized by endocytosis, PCI has the potential to overcome off-target cytotoxicity of such treatments. We have recently discovered that PCI of the gelonin based EGFR-targeting toxin rGel/EGF is highly effective in the cetuximab-resistant HNSCC cell line SCC-040 (Fig 4A) [59]. The PCI-induced efficacy of rGel/EGF is comparable to that observed in another HNSCC cell line (SCC-026) sensitive to cetuximab treatment (Fig. 4A). Intracellular and nuclear pools of EGFR has further been associated with cancer treatment resistance [60-62]. These pools are subjected to endosomal trafficking prior to nuclear translocation which may represent targets for PCI in order to overcome resistance. The time frame of disrupted endocytic vesicles following PCI is, however, limited (~6 hrs) [63] and the effect of these EGFR-related resistant mechanisms will therefore probably be transient and of little clinical relevance. PCI of the HER2-targeted toxin MH3-B1/rGel has been shown to exert similar efficacy in the trastuzumab-resistant SKOV-

3 cell line as in the trastuzumab-sensitive SK-BR-3 cells (Fig. 4B) (unpublished results). PCI may therefore represent a targeted treatment modality in cases where the cells are no longer responsive to antibodies or TKIs aimed at a specific target. A promising indication here is ovarian cancer, where HER2-targeted TKIs and mAbs have failed to demonstrate any clinical benefit despite HER2 being over expressed in up to 30 % of the cases [64].

The high selectivity together with the major toxicity provided by the toxin-moiety of the targeting toxin is at least partly the reason why PCI is able to induce targeted toxicity also in cancers with low target expression. This was shown in the triple negative breast cancer (TNBC) cell line MDA-MB-231 which, despite its low HER2 expression and reported poor response to HER2 targeted therapy, responded surprisingly well to PCI of MH3-B1/rGel [65]. Low expressing HER2 cells are generally not dependent on HER2 signaling. This has been discussed to have implications for HER2 internalization rate where HER2 low and non-dependent cell lines exert a more rapid HER2 endocytosis compared to HER2 high and dependent cell lines [66-68]. We therefore believe that increased level of HER2 endocytosis and subsequent MH3-B1/rGel uptake in the HER2 low expressing MDA-MB-231 cell line contributes to the high PCI efficacy. These results demonstrate the relevance of targeted drug delivery in systems where the target is unsuited as a pharmacological target it selves. The cellular response to PCI of MH3-B1/rGel has been reported to correlate positively to HER2 expression demonstrating HER2 specificity [65]. The high efficacy in HER2 low-expressing cancers may also be beneficial for the treatment of tumors with heterogeneous HER2 expression and also in TNBC where effective treatment modalities are highly warranted.

We have also shown that PCI of the CD133-targeting immunotoxin AC133-saporin is a specific and efficient strategy to kill cancer stem-like CD133^{high} WiDr colorectal cancer cells resistant to PDT, chemotherapy and radiation [69]. In addition, androgen therapy resistant DU145 cells over expressing CD44 are although PDT resistant, highly sensitive to PCI of the CD44-targeting immunotoxin IM7-saporin [70]. For a review on PCI-based targeting of cancer stem cells we refer to Selbo et al, PPS 2015)

3.5 PCI efficacy; method of assessment

As described above, PCI has been shown as an effective treatment modality in several models of resistant cancers. These studies have also indicated PCI to have similar or even increased efficacy in resistant cancers compared to non-resistant models. Care must, however, be taken when comparing the effects of PCI in cellular systems.

PCI may be regarded as a combination therapy between the photochemical treatment and the drug of interest, and comparison of the quantitative effects in different cell lines are often difficult due to variations in sensitivity not only to the macromolecular drug of interest but also to the photochemical treatment. In cell lines with similar sensitivity towards the macromolecular drug but with different sensitivity towards the photochemical treatment (Fig. 5A and 5A1)), PCI efficacy may be assessed by the following formula [71]:

Equation i: $$\boldsymbol{PCI\ efficacy = \frac{LD_{50}\ (Photochemical\ treatment)}{LD_{50}\ (PCI)}}$$

Where LD_{50} represent the light dose, as measured in seconds exposure time, needed to kill (or reduce the viability of) 50% of the cells.

However, comparing the efficacy of PCI in cell lines with different sensitivity to the macromolecular drug in addition to the photochemical treatment (Fig. 5A and 5A2), the formula must be corrected to [65] :

Equation ii: $$\boldsymbol{Drug-corrected\ PCI\ efficacy = PCI\ efficacy\ x\ IC_{50}\ (drug)}$$

Where IC_{50} is the macromolecular drug concentration, as measured in µM, which inhibits 50 % of the biologic activity of its target or reduces the cell viability to 50 %.

The efficacy of a targeting drug (without PCI) is often represented by the targeting index (TI) assessed by;

Equation iii $$\boldsymbol{Targeting\ Index\ (TI) = \frac{IC_{50}\ (non-targeted\ drug)}{IC_{50}\ (targeted\ drug)}}$$

Subsequently, the efficacy of PCI of a targeting drug (Fig. 5B) may be assessed by calculating the PCI targeting index;

Equation iv $$\boldsymbol{PCI\ Targeting\ Index\ (TI)} = \frac{\boldsymbol{IC_{50}\ (non\text{-}targeted\ drug\ with\ PCI)}}{\boldsymbol{IC_{50}\ (targeted\ drug\ with\ PCI)}}$$

at a set light dose. PCI TI is dependent on the fraction of biologic inhibition/reduction of cell viability of interest and may increase with increased toxicity of the treatment.

In summary, assessment of PCI efficacy in resistant cell lines should be based not only on the increased cytotoxicity observed when adding the macromolecular drug of interest to the photochemical treatment but also on the cellular sensitivity to the macromolecular drug itself.

4. Drug delivery systems to overcome cancer resistance

PCI represents a unique cancer-targeted cytosolic drug delivery system and has little shared properties with more classic drug delivery systems (DDS) such as nanoparticles, liposomes and polymers. Drugs suited for administration with PCI have low potency in cancer treatment both as monotherapy and in combination with other classical DDS due to endocytic trapping. These hydrophilic drugs with relatively high molecular weight are not substrates for the drug efflux pumps and are therefore not likely to be affected by mechanisms for MDR. In addition, the high specific toxicity provided by PCI is the reason why only one or very few treatments are expected to be sufficient to obtain complete response, and both the completed and ongoing clinical trials are designed with only one PCI treatment. The high effect obtainable by only few treatments should, in addition, limit the possibilities for acquired resistance towards the treatment.

Classical DDS have, however, also been used to overcome cancer resistance [20]. These studies have mainly been focused on multiresistant cancers with the aim to inhibit drug efflux pumps. Several compounds have been suggested as candidate drugs for inhibition of such pumps including verapamil, diltiazem and cyclosporine A [72, 73]. Formulation of efflux pump inhibitors in nanoparticles with cancer targeting

moieties on the surface may overcome problems with low specificity and several groups are aiming for the development of such formulations for clinical use [20]. DDS including PCI may also provide possibilities for treatment with nucleic acid-based drugs to target MDR protein expression [74, 75]. Clinical use of DNA and RNA is, however, still limited by insufficient transfection- and transduction-efficacy as well as high toxicity.

Cathepsin- and proteolytic- cleavable linkers have been utilized in targeted cancer therapeutics such as in the antibody-drug conjugate (ADC) brentuzimab vedotin approved for the treatment of Hodgkin lymphoma and systemic anaplastic large cell lymphoma [76, 77]. The targeting moiety (CD30-antibody) and an antineoplastic agent (monomethyl auristatin) are in this ADC connected through a cathepsin-cleavable linker. When brentuzimab vedotin is taken up in CD30 expressing cells it accumulates in endocytic vesicles in which the linker between the antibody and cytotoxic moiety is cleaved. The cytotoxic moiety is relatively small and lipophilic and is after separation from the antibody free to diffuse across the membrane of the endocytic vesicle into the cytosol where it can exert its effect. The adverse effects of brentuzimab vedotin is mainly thought to be related to *(1)* diffusion of free active drug from the target cells into the extracellular space where it can act on off-target cells, *(2)* release of the active drug in the extracellular space, *(3)* unspecific uptake of the drugs through its antibody Fv-fragment or *(4)* uptake of the drug in normal cells with target expression. These listed mechanisms of adverse effects are also thought to apply for trastuzumab emtasine, another ADC with similar mechanism of action as brentuzimab vedotin approved for the treatment of metastatic breast cancer[76, 78]. Both brentuzimab vedotin and trastuzumab emtasine have been shown effective in resistant cancer[79] and PCI of a targeted toxin clearly shares the mechanistic step of endosomal release with these ADCs. The profile of adverse effects following PCI of targeted toxins should, however, not be comparable to that of the mentioned ADCs. This is mainly due to the difference in structure and physio-chemical properties of the active cytotoxic drugs in these two systems. Both brentuzimab vedotin and trastuzumab emtasine comprise relatively small, lipophilic and highly cytotoxic drugs which once set free from the antibody part of the ADC is able to diffuse freely across cellular membranes. The targeted toxins utilized for PCI delivery is, on the other hand, based on type I ribosome inactivating protein toxins (RIPs) which, due to their hydrophilic properties and large size, are unable to cross cellular membranes [80, 81]. These toxins are therefore

dependent on destabilization of the endocytic vesicles to exert their therapeutic effect and are, in the absence of such endosomal destabilization, associated with low cytotoxicity due to lysosomal degradation [80-82]. Further, the cancer targeting properties of brentuzimab vedotin and trastuzumab emtasine are mainly provided by the CD30- and HER2- antibody parts, respectively. Endo/lysosomal cathepsins and proteolysis is a common characteristic for mammalian cells and these ADCs therefore exert their effects in every cell of which they are taken up including target cells, target expressing normal cells and normal cells with non-specific uptake. This is in contrast to PCI of targeted toxins, which, in addition to the selectivity provided by the targeting moiety, exerts cancer selectivity through the PS as well as the confined light exposure (see chapter 3). Overall, the 3-fold selectivity provided by PCI of targeted toxins together with the utilization of a targeted toxin with minor toxicity without endo/lysosomal destabilization is the reason why off target toxicity (dark toxicity) should be minor compared to ADCs as described above, and PCI of a targeted toxin should subsequently be considered as a better alternative." TPCS2a, the clinical relevant PS photosensitizer has not been shown to induce any dark toxicity [23].

5. Conclusion

Intrinsic and acquired resistance towards cancer therapeutics is one of the major limitations for cancer cure. The mechanisms of cancer resistance are numerous and often orchestrated to inhibit the effect of drugs with different structures and mechanisms of action. PCI is a DDS designed for cytosolic translocation of hydrophilic macromolecules. These macromolecular drugs are not affected by the most common mechanisms of cancer drug resistance and PCI therefore offers a method for circumvention of cancer resistance. PCI can also be applied for the delivery of targeting toxins and such treatment has been shown to overcome resistance to therapeutic antibodies towards the same target. PCI of targeted toxins has further been shown to exert high target-specific toxicity also in models with lower target expression indicating this treatment modality as an effective approach for targeted therapy in solid cancers with heterogeneous target expression. A ROS-resistant cancer model has, in addition, been shown sensitive to treatment with PCI, despite that ROS induction is an important step of PCI-induced cytosolic delivery.

The studies reviewed here indicate PCI as a treatment modality for drug-resistant cancer (Fig. 6) including MDR, and preclinical evaluation in relevant *in vivo* models is clearly warranted to explore this further.

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FIGURE LEGENDS

Fig. 1: Mechanisms of resistance to anticancer therapeutics.

Fig. 2: Mechanism of PCI. The drug of interest is taken up in the cell by means of endocytosis and accumulates in endocytic vesicles together with the amphiphilic PCI relevant photosensitizer (PS). Light exposure activates the PS and induces generation of ROS which disrupt the endo/lysosomal membrane and thereby release the entrapped drug. The drug can then interact with its target and induce cytotoxicity.

Fig. 3: PCI-induced reversal of doxorubicin resistance.
A: Doxorubicin (red circles) is distributed to the endo/lysosomal compartments in the doxorubicin resistant MCF-7/ADR cell line compared to **B:** the sensitive MCF-7 cells where doxorubicin is localized to the nucleus. **C:** PCI disrupts the membranes of the endocytic vesicles and induces cytosolic release of doxorubicin which subsequently localize to the nucleus and exerts its therapeutic effect (C). The arrows indicate the transport direction of doxorubicin.

Fig. 4: PCI of EGFR- and HER2-targeted toxins in cancers resistant to EGFR- and HER2-targeted antibodies. A: SCC-026 and SCC-040 HNSCC cell lines (EGFR-positive) were treated with 660 nM cetuximab (Cetux) for 6 days or PCI of 0.1 nM rGel/EGF using a dose of photosensitizer and light that reduces viability by approx. 50 %. **B:** SK-BR-3 and SKOV-3 cells (HER2-positive) were treated with 156 nM trastuzumab (Trast) for 3 days or PCI of 2 nM MH3-B1/rGel using a dose of photosensitizer and light that reduces viability by approx. 60 %.

Fig. 5: Comparison of PCI effect in different cell lines. A and A1: In cell lines with different sensitivity to the photochemical treatment but with similar response to the drug PCI efficacy can be established by comparing the difference in light dose ratio between LD_{50} for PCI and the photochemical treatment (Equation i). **A and A2:** In cell lines with different sensitivity to both the photochemical treatment and the drug, PCI efficacy must be corrected for drug sensitivity as measured by IC_{50} in both cell lines (Equation ii). **5B:** For PCI of targeted drugs a PCI targeting index can be established by comparing the difference in IC_{50} ratio between the non-targeted drug and the targeted drug in combination with PCI (Equation iv). LD_{50}: Letal dose in

seconds of light exposure needed to kill or reduce the viability of 50% of the cells.
IC_{50}: Inactivation concentration in μM needed to kill or reduce the viability of 50% of the cells.

Fig.6: PCI as treatment of resistant cancer; current documentation.

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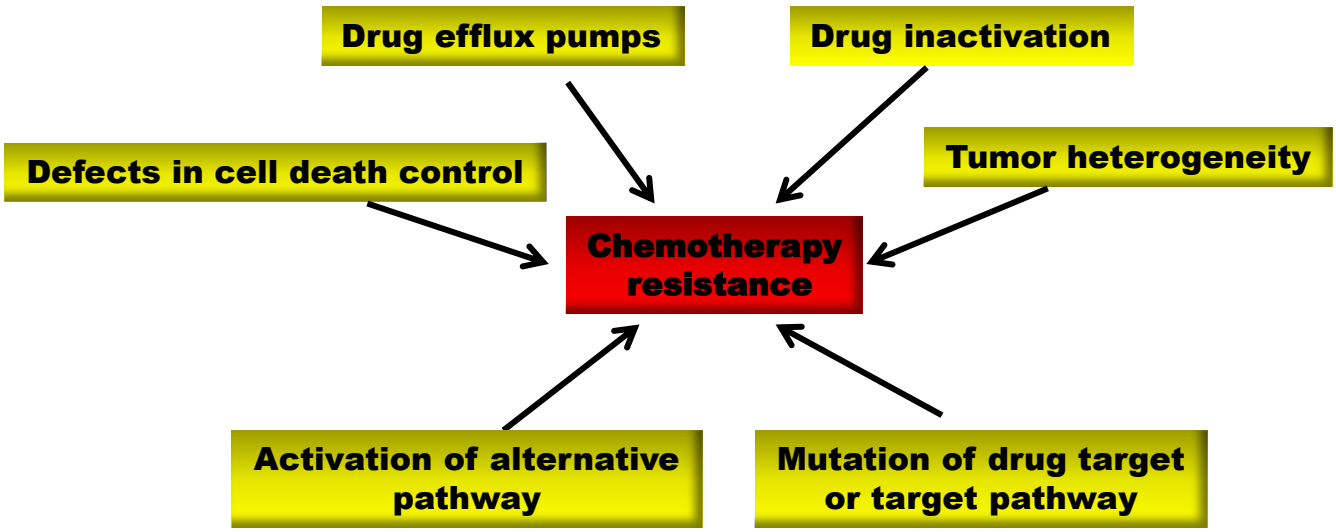
Fig. 1



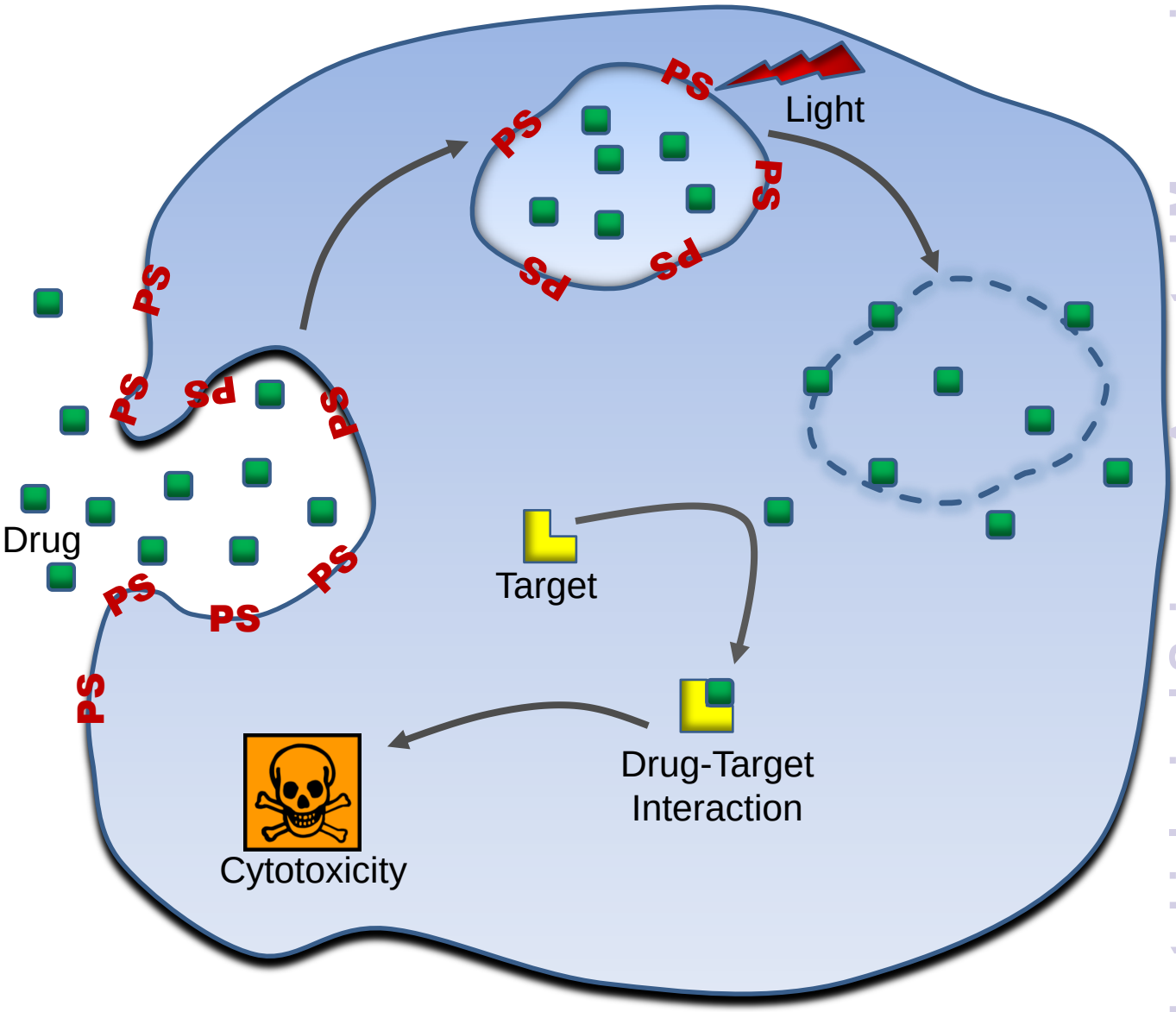
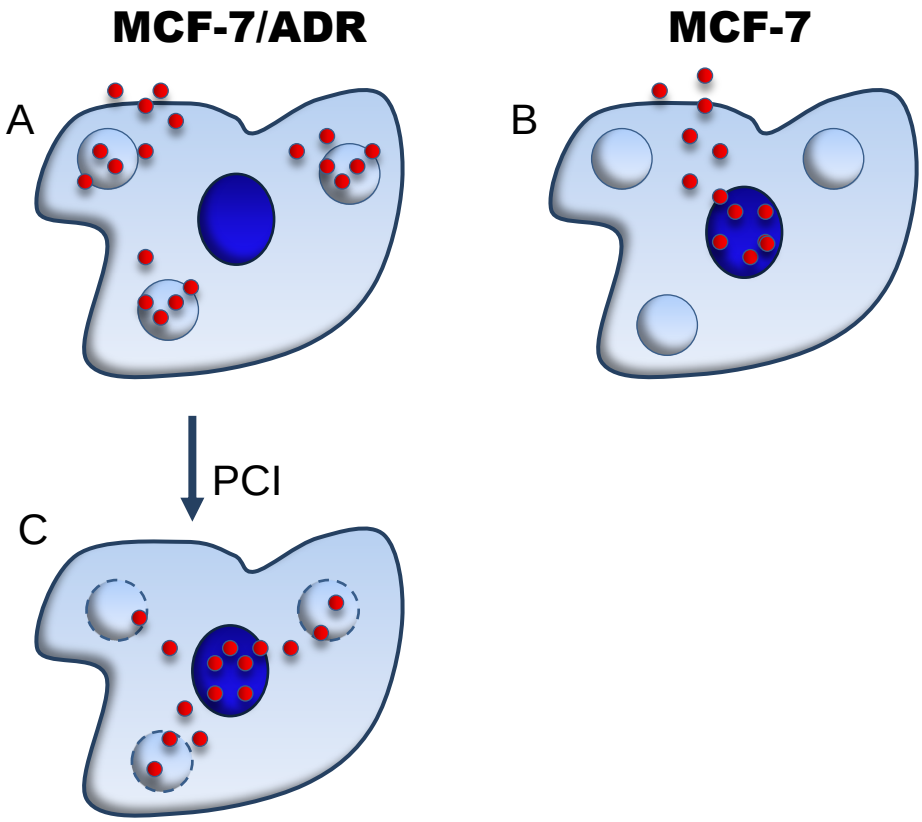
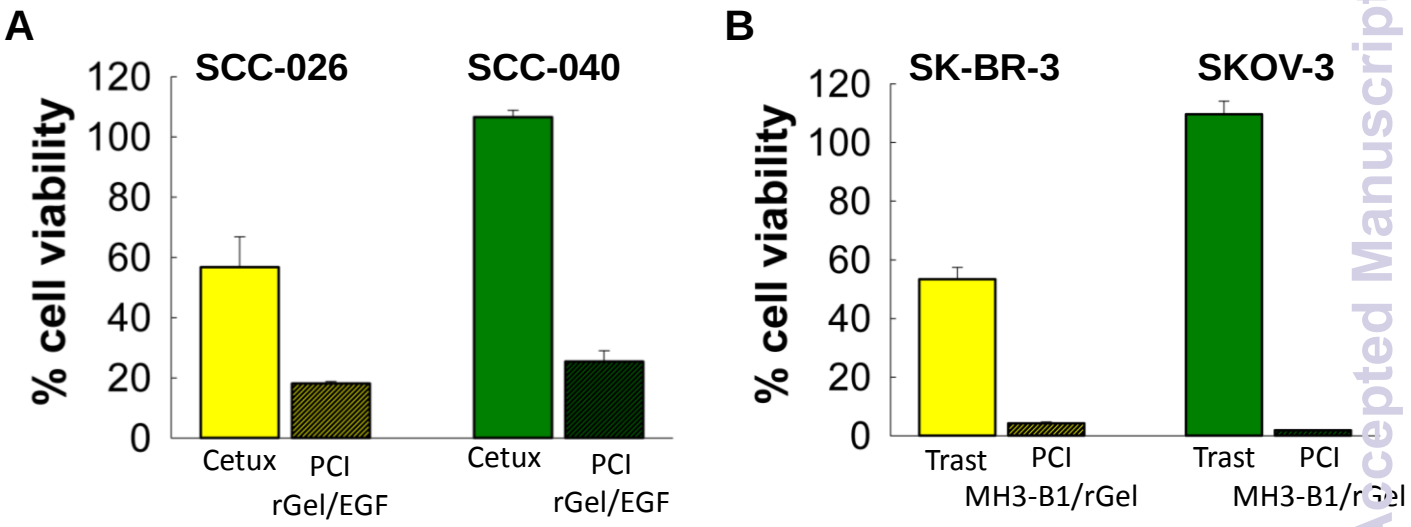


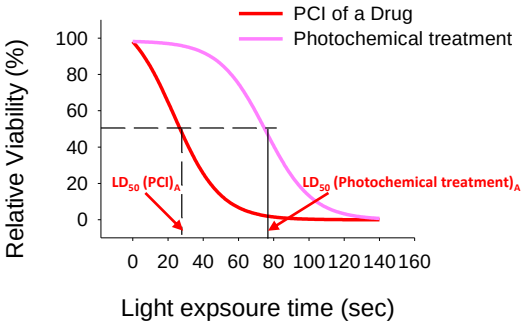
Fig. 3



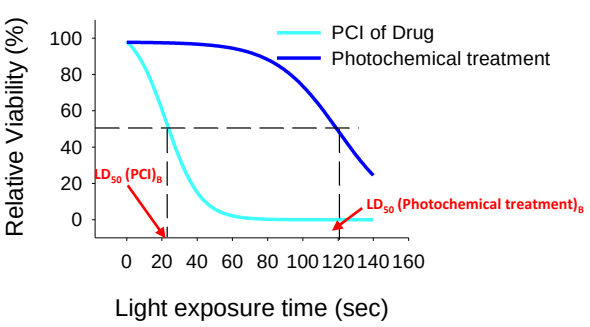


A

PCI of Drug in cell line A
Sensitive to Photochemical treatment

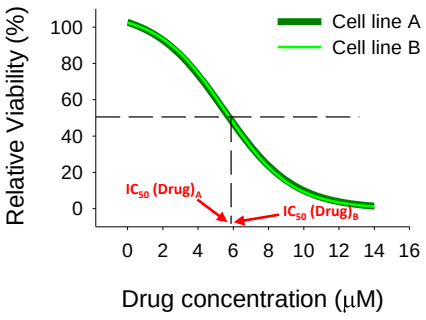


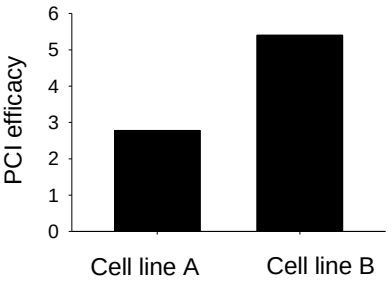
PCI of Drug in cell line B
Resistant to Photochemical treatment



1.

Drug Sensitivity in cell line A and B

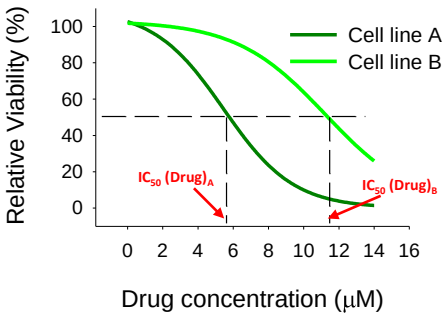


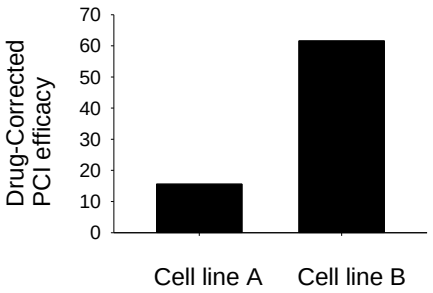


$$PCI\ efficacy = \frac{LD_{50}\ (Photochemical\ treatment)}{LD_{50}\ (PCI)}$$

2.

Drug sensitivity in cell line A and B

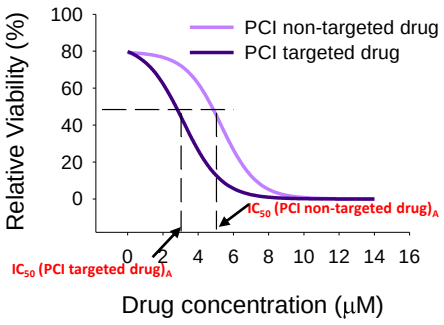




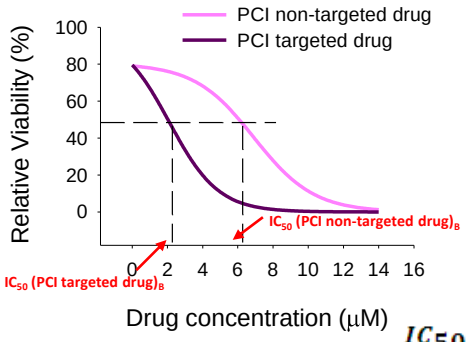
$$Drug-corrected\ PCI\ efficacy = PCI\ efficacy\ x\ IC_{50}\ (drug)$$

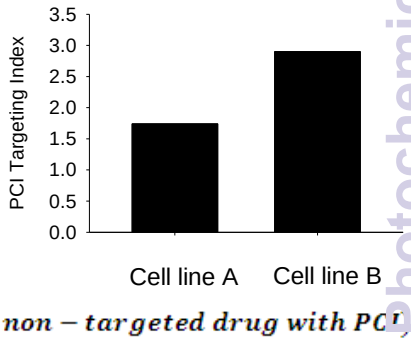
B

PCI of targeted drug in cell line A



PCI of targeted drug in cell line B





$$PCI\ Targeting\ Index\ (TI) = \frac{IC_{50}\ (non-targeted\ drug\ with\ PCI)}{IC_{50}\ (targeted\ drug\ with\ PCI)}$$

PCI-induced resistance circumvention

- **PCI releases hydrophilic macromolecular drugs which are not subjected to drug efflux.**
- **PCI induces site-directed targeted toxicity in systems with low target expression.**
- **PCI is effective in ROS- and radiation-resistant cells.**
- **PCI induces targeted toxicity in systems resistant to other targeted therapies.**
- **PCI photosensitizers are not subjected to drug efflux.**
- **PCI can counteract resistance caused by alteration of drug sequestration**